JAPANESE JOURNAL OF BOTANY

PUBLISHED BY NATIONAL RESEARCH COUNCIL OF JAPAN

Vol. X No. 3

(Published December 31, 1939)

CONTENTS

TRANSACTIONS

N.B.—No Abstracts in this No.

Some considerations on the classification of *Oryza sativa* L. into two subspecies, so-called '*Japonica*' and '*Indica*'

By Hirosi TERAO and Usaburô MIDUSIMA
Imperial Agricultural Experiment Station

With 10 figures and 21 tables

(Received July 19, 1939)

Introduction

On finding the fact that some rice varieties of tropical origin were remarkably resistant to blast or rotten-neck (*Piricularia oryzae* CAV.), the senior author had started about 20 years ago at the Imperial Agricultural Experiment Station, Nisigahara, Tôkyô, crossing experiments between Japanese and tropical varieties with the object of transferring the resistance of the latter to the former. In conducting the experiments, however, it first came to his attention that nearly all of the F_1 hybrids raised were considerably sterile displaying in their succeeding generations various degrees of sterility, ranging from complete sterile to full fertile, as if they would have originated from species hybridizations. This made him to give up, for the time, the prime purpose for obtaining blast-resistant varieties and to intend to make out how the sterility was caused. The study has been carried on by him intermittently for about 10 years until 1934 during which time an extensive series of crossing have been made. Nevertheless, he could not obtain even a possible clue to the explanation of the origin of sterility prevented by the following limitations. As described later the F_1 hybrids obtained in these crosses are usually later in heading than any of their parental varieties and in ordinary field culture this, being accompanied by their increased vegetative vigor (heterosis), led some of them frequently left unheaded or to head in later part of autumn when it is so cool and unfavorable for their blooming and fertilization. Consequently their degrees of sterility (or fertility) were variable influenced by the climatic conditions of the year in which they were cultivated. Since the true degree of genetical sterility was disturbed physiologically or mechanically affect-

ed by the environmental conditions, it was quite difficult for him to secure accurate quantitative data as to the sterility in each of the F_1 hybrids and of their later progenies, which might serve as the primary clue to solve this problem.

In 1934 he has restarted the study at Kônosu Station of the Imp. Agr. Exp. Stat. with the cooperation of the junior author with renewed plan and materials. Here every measures have been taken for controlling the surplus vegetative growth of hybrid plants and inducing their time of heading earlier so as to make them bloom in favorable climatic conditions without any harmful influences on their physiology. As a criterion to be based upon for determining their sterility in a more stable and accurate manner, the degree of abortion in their sexual cells was taken. This, together with their degree of actual seed setting in favorable environmental conditions, enabled the authors to characterize the sterility in each of the crosses fairly accurately.

Thus by the renewed method of study followed in these five years several unknown parts of the problem have been made clear, but the underlying cause of the sterility has yet been left unexplained, which is considered to be extremely complex and not to be subjected to simple explanation. The data accumulated in these years, however, seem to suggest that the classification of *O. sativa* L. into two subspecies, '*Japonica*' and '*Indica*', made by KATÔ and his collaborators (1930) from the morphological and serodiagnostic stand-points as well as from the sexual affinity, can not readily be accepted. The present paper aims to present a part of the experimental results which leads the authors to such a conclusion as above.

Part I. Sexual affinity among the varieties used

KATÔ and others (1928, '30) have found in their crossing experiments two different groups in rice varieties cultivated in the main rice-producing regions in the world. The members of each of the two groups are characterized firstly by their inability to form fertile F_1 hybrids with those of the other. Basing upon this fact, together with the morphological and serodiagnostic differences, they have regarded the two groups as subspecies and have proposed to name one '*O. sativa* L. subsp. *Japonica* KATÔ' and the other '*O. sativa* L. subsp. *Indica* KATÔ'. Thus they have classified tentatively many varieties of rice collected by them from Japan Proper, Korea, Formosa, China, Java, India, the United States of America, etc., into '*Japonica*' and '*Indica*' and have reached the conclusion that the rice native to Japan Proper, Korea, and Northern China all belong to '*Japonica*' and that found in Formosa,

Southern China, Java, India, Ceylon, etc., to '*Indica*', while some of the varieties in Central China, Hawaii, and the United States of America to '*Japonica*' and others to '*Indica*'. This has been supported by JONES (1930) who has obtained quite the same results as those reported by them in his crossing experiments with Japanese and American varieties conducted in California, U.S.A.

In the crosses made by the authors among the varieties including also those native to Japan Proper, Formosa, Indo-China, Java, India, and the New World, however, the sexual affinity measured by the degree of sterility in F_1 hybrids seems not to be an adequate criterion by which they can be divided distinctly into two groups. The intricate relationships exhibited in their mutual affinity are given particular attention in this Part.

I. MATERIAL

Among a great many varieties comprising those native to Japan Proper and various rice-producing regions in the world, each of which has been continued with careful management at Kônosu Station, the authors have taken 26 varieties as parents in their crossing experiments. These, consisting of 12 Japanese and 14 foreign varieties, have been selected each to have some distinctive phenotypical traits so as to be proper to study not only their mutual affinity, but the behavior of these in their hybrid offspring. Their name and origin may be reviewed below.

Varieties native to Japan Proper	Varieties of foreign origin	
J_1 : Kuroka Moti	NA_1 : Carolina	North America
J_2 : Murasaki Ine	NA_2 : Hondulas	,,
J_3 : Sen'iti	SA : Jaguary	South America
J_4 : Kamezi	Jv_1 : Loktjan	Java
J_5 : Sekiyama	Jv_2 : Ketan Nangka	,,
J_6 : Sekitori	I_1 : Surjamkhi	India
J_7 : Sinriki	I_2 : Bason Takakal	,,
J_8 : Omati	I_3 : Mushakdanti	,,
J_9 : Aikoku	I_4 : Karalath	,,
J_{10} : Kameno-o	I_5 : Charnack	,,
J_{11} : Kokuryômiyako	I_6 : Danahara	,,
J_{12} : Tôgô	IC : Te-tep	Indo-China
	F : Oka-Ine	Formosa
	H : Hawaii No. 154	Hawaii

Among these varieties crosses comprising 140 combinations have been made in which 20 pairs of reciprocal ones are included as shown in the following chart, Fig. 1.

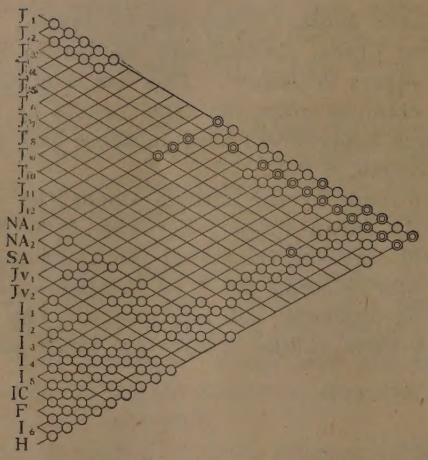


Figure 1. Chart illustrating the cross combinations among the varieties used. ○ and ◎ indicate one side and reciprocal matings respectively.

II. Preliminary Experiments

As referred to before, crosses between the varieties native to Japan Proper and those of foreign origin result, with almost no exceptions, in hybrids showing remarkable lateness in heading, regardless whether

Table I. Dates of heading of some parental varieties and their F_1 hybrids in ordinary paddy field culture. (sown on 10/V ; transplanted on 1/VII)

Parental varieties	Date of heading	F_1s	Date of heading
J_1	21/VIII	$J_1 \times NA_1$	26/IX
J_2	8/IX	$NA_2 \times J_1$	25/IX
J_3	28/VIII	$Jv_1 \times J_1$	25/IX
J_4	8/IX	$Jv_2 \times J_1$	8/X
J_5	10/VIII	$I_1 \times J_1$	23/IX
NA_1	8/IX	$J_1 \times I_3$	30/IX
NA_2	5/IX	$J_1 \times IC$	5/X
Jv_1	11/IX	$J_1 \times F$	4/IX
Jv_2	15/IX	$J_1 \times H$	4/X
I_1	28/VIII	$J_3 \times NA_1$	20/IX
I_3	8/IX	$J_4 \times NA_1$	25/IX
IC	10/IX	$J_5 \times NA_1$	22/VIII
F	28/VIII		
H	4/IX		

they are full fertile or sterile. It, therefore, necessarily follows that in ordinary method of culture their degree of seed setting is apt to be disturbed physiologically affected by the unfavorable cool climate in later part of autumn, making it difficult to characterize accurately the dgeree of sterility in each of the cross combinations.

To eliminate the physiological disturbances and to secure stable data for the purely genetical sterility of the F_1 hybrids the authors have conducted some preliminary experiments during three successive years using sets of F_1 hybrids, each of which includes five individuals propagated vegetatively from respective F_1 mother stocks reserved throughout these years. Here the hybrid plants were planted in WAGNER's pots with controlled manure in order to manage them with utmost care and to avoid surplus vegetative growth leading them physiologically to their delayed heading. For controlling their heading earlier so as to make them bloom under favorable climatic conditions artificial shortening of day length was undertaken. After their heading they were protected in a glass house safely from any mechanical damages in case of stormy weathers. From the results of these experiments the authors have confirmed the following facts:

i. By applying properly the short day method the F_1 hybrids can be induced to bloom in favorable mid-summer climate, where their degrees of seed setting are observed to be markedly higher than those in ordinary paddy field culture. Here genetically fertile hybrids which have displayed variable degrees of false sterility under unfavorable conditions manifest their full fertility with more than 90% seed setting and each of partially sterile ones shows that in a definite degree which is almost invariable throughout the three experimental years. In rare cases, however, a few of the hybrids show under short day treatment considerably less degree of seed setting than in the case when they are untreated. Close investigations have revealed that in such hybrids anther walls become conspicuously hardened insomuch that they are unable to dehisce at the time of blooming supplying no pollen grains to the stigma.

ii. Under microscope the parental varieties show nearly perfect pollen grains with a few presumably abortive ones which are morphologically imperfect or degenerated, amounting to, in most cases, 2–5% of the total; whereas the F_1 hybrids give various degrees of pollen abortion, ranging, from 0 to almost 100%, which are considered to be the characteristics of the respective cross combinations and are noted to be invariable whether they undergo the short day treatment or not.

iii. The degree of pollen abortion displayed in each of the F_1 hybrids corresponds usually to the degree of embryo-sac abortion. In some cases, however, they do not agree with each other. In such a case the former is observed to be always higher than the latter. It can

be easily concluded from the fact that under short day treatment such a hybrid shows far higher a degree of seed setting than its pollen fertility.

iv. All of the parental varieties have 24 somatic and 12 meiotic chromosomes. Owing to the minuteness of the somatic chromosomes the authors failed to detect any morphological differences between the two

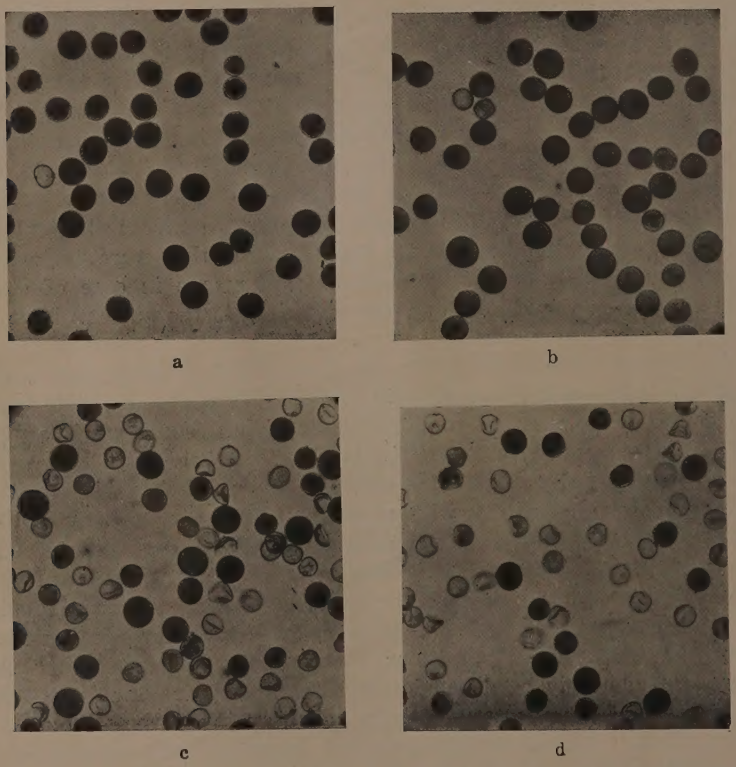


Figure 2. Microphotographs showing various degrees of pollen abortion found in the F_1 hybrids. a, $J_1 \times NA_1$. b, $J_1 \times I_3$. c, $J_2 \times I_6$. d, $NA_2 \times I_6$

parental sets whose collaboration results in the formation of a sterile F_1 hybrid. In each of the sterile F_1 hybrids investigated the microsporogenesis is carried out apparently regularly and the normal tetrad formation follows. In spite of that, degeneration of microspores takes place in various degrees before they develop into matured pollen grains. From the paralellism in the degree of abortion between the pollen and the embryo-sac, it is considered that in the ovules the macrosporogenesis

follows a similar course. The situation in the degeneration of sexual cellls here observed accords fully with what was reported by KATÔ and others in the sterile F_1 hybrids raised by them in the crosses *'Japonica'* × *'Indica'*.

Hence, it may be appropriately considered i) that in most cases the short day treatment does not cause any harmful physiological in-

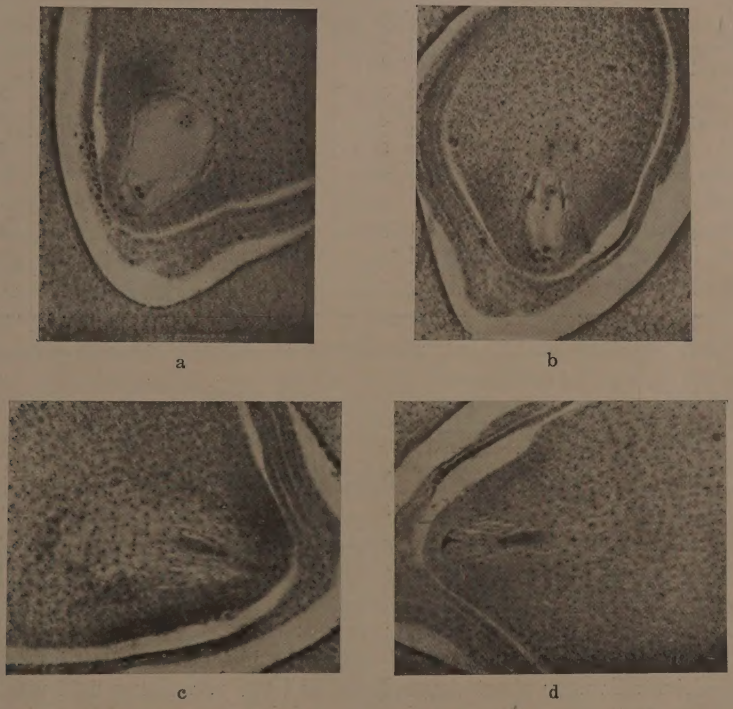


Figure 3. Microphotographs showing aborted embryosacs found in the F_1 $J_1 \times H$. a, a normal embryosac with an egg apparatus, two polar nuclei, two synergids, and a part of the divided antipodal cells. b-d, abnormal ones which have ceased their development at various stages.

fluences upon the vegetative as well as the generative growth of the F_1 hybrids, but promotes their degree of seed setting elimianting the physiological sterility caused by the unfavorable climatic conditions and leading them to display the degree of sterility or fertiliy closely approximate to that from purely genetical cause; and ii) that the degree of pollen abortion shown in the F_1 hybrids can be served, in most cases, as the index of their degree of embryo-sac abortion, the latter is the most stable

TABLE II. Percentage of normal pollen, normal embryosac, and seed setting in some of the hybrids under short day as well as untreated condition (1935).

F_1s	Date of heading		%-age of normal pollen		%-age of normal embryo-sac		%-age of seed setting	
	treated	un-treated	treated	un-treated	treated	un-treated	treated	un-treated
$J_1 \times$ IC	20/VIII	5/X	50.4	49.0	48.1	51.3	40.0	0.0
(The reciprocal)	,,	,,	47.1	49.8	41.8	47.6	40.8	0.0
$J_1 \times$ F	18/VIII	4/IX	56.1	50.1	50.2	52.8	36.7	28.0
(The reciprocal)	19/VIII	,,	46.2	51.2	52.1	49.5	44.5	20.0
$J_2 \times$ SA	17/VIII	18/IX	91.5	92.0	96.4	97.5	94.0	60.0
(The reciprocal)	,,	,,	92.3	95.1	98.0	95.4	88.0	56.5
$J_3 \times$ NA	21/VIII	20/IX	93.0	95.0	95.4	96.1	92.1	49.1
(The reciprocal)	,,	,,	95.4	96.3	93.5	97.5	93.6	48.9
$J_1 \times$ H	17/VIII	4/X	3.0	4.6	43.5	45.1	8.0	0.0
(The reciprocal)	,,	,,	2.0	0.0	51.3	46.5	2.0	0.0

criterion for determining the true degree of sterility, but so tedious and time consuming to investigate.

III. METHODS

From the above-mentioned facts the authors have employed the following methods for their study:

i. All the materials were planted individually in 1/5,000-are WAGNER's pots with controlled manure so as to be convenient for applying the short day method and for protectng them in a proper place from any mechanical damages caused by stormy weathers.

ii. Each F_1 set consisted of about 10 individuals, half of which was cultivated under short day method and the remaining half was left untreated as the control.

iii. For applying the short day method, which began after the fifth leaf of F_1 seedlings had fully grown and was continued during about 30 days, large boxes of galvanized sheet-iron with a ventilator, having the basic area of about 6 feet square and the height of 4 feet, were used, which were capable of covering 49 pots at a time. The F_1 seedlings were exposed to light from 6 A.M. to 6 P.M. during the treatment.

iv. For determining the degree of sterility of each F_1 set the percentage of normal pollen (or embryo-sac) was taken as the chief criterion and the degree of seed-bearing under short day as well as untreated conditions was also taken for checking. The methods for determining these are as follows.

Percentage of normal pollen:

At the period when the hybrids are about to bloom five flowers are voluntarily taken from each three individuals of respective F_1 sets, the anthers of which are examined with aceto-carmine under microscope. After the flowers have been confirmed each to show about the same degree of pollen abortion, the counting of normal pollen is made on any one of them, where pollens of imperfect shape, those lacking content, and those very slighly coloured with the dye are regarded to be abnormal and nonfunctional. Of more than 2,000 grains the percentage of normal pollen is calculated.

Percentage of normal embryo-sac:

At the time of heading five of the fully developed ears are taken from any one of the individuals of the respective F_1 sets which have been already confirmed to show the same degree of pollen abortion. They are fixed wholly with CARNOY's fluid and reserved in 85% alcohol. The ovaries are carefully teased out with a forcep from each ear separately and are made into permanent preparations by the paraffin method. The sections are cut at the thickness of 25 microns and are stained with iron-alum haematoxylin. In investigating the embryo-sac under microscope, fully developed ones with perfect generative and vegetative cells, i.e., an egg-apparatus, two polar nuclei, a mass of the divided antipodal cells, and two synergids (frequently obscured) are taken to be normal and the remainders, consisting of incompletely developed and degenerated ones, such as those which have ceased their development after the formation of megaspore tetrad and remain as such, those in which the division and differentiation of the nuclei have not yet been completed, and those which have degenerated presumably in the tetrad stage and the nucellus has been filled with surrounding tissue leaving some nuclear substance stainable with haematoxylin, are taken to be abortive.

Percentage of seed setting:

The calculation is made on each five ears of the individuals forming the respective F_1 sets under short day treatment. In a few hybrids which display remarkable depression in seed setting under short day condition, the calculation is made on the untreated lots whose blooming and fertilization are performed under glass house condition, being fended off the climatic influences upon these.

v. After the harvest the F_1 stubbles, each one from the respective sets, are transplanted in a green house and are reserved through the winter until the next spring when they are divided into necessary numbers and are used for further investigation.

IV. Experimental results

1) *Compatibility among the varieties used*

In conducting the crossing experiments no noticeable difference in compatibility was noted among the varieties used. They crossed easily with one another regardles whether the resulting F_1 hybrids were fertile or sterile. Certation was also inconceivable. By applying the stigma of a variety with a mixture of pollen grains consisting of two different kinds, each of which give rise to fertile and sterile hybrids respectively, the authors obtained these in the ratio approximately 1:1, as shown in the following table.

Table III. Results of crossing experiments with mixed pollens

Pistillate parents	Constitution of mixed pollen applied	No. of flowers pollinated	No. of seeds obtained	No of F_1 hybrids obtained		No. of selfed individuals	Ratio
				Sterile	Fertile		Sterile : fertile
I_2	$I_1 + J_3$*	237	142	67	72	3	1 : 1.07
I_5	J_1*+ I_1	194	78	40	38	0	1 : 0.95
J_2	$J_1 + F$*	250	110	59	51	0	1 : 0.86
J_4	$J_2 + I_6$*	270	163	75	86	1	1 : 1.14

* shows the pollen parents forming sterile hybrids with the respective pistillate ones.

Table IV. The percentage of normal pollen and seed setting of the parental varieties under short day treatment

Varieties native to Japan Proper	%-age of normal pollen	%-age of seed setting	Varieties of foreign origin	%-age of normal pollen	%-age of seed setting
J_1	98.3	94.0	NA_1	96.4	98.1
J_2	96.2	95.2	NA_2	97.6	96.5
J_3	98.8	94 3	SA	98.7	94.3
J_4	97.7	93.2	Jv_1	94.6	93.6
J_5	97.5	94.5	Jv_2	96.8	90.0
J_6	99.0	96.3	I_1	97.5	89.5
J_7	98.1	91.2	I_2	98.8	94.2
J_8	96.4	93.5	I_3	96.5	90.5
J_9	98.6	96.1	I_4	99.0	95.1
J_{10}	95.5	98.0	I_5	95.4	91.7
J_{11}	97.4	92.1	I_6	95.4	92.3
J_{12}	98.7	93.7	IC	97.0	92.7
			F	97.5	97.5
			H*	91.2	91.1

* untreated.

2) *Fertility of the parental varieties*

The varieties here used as cross parents show in ordinary paddy field culture usually 70 to 90% seed setting, but a few of them of foreign origin which are relatively later in heading than the others give frequently that less than 50%. Under the short day treatment, however, all of them manifest their highest degree of seed setting more than 90%, except the one, Hawii no. 154, which shows about 30%, far less the degree of seed setting than that under ordinary cultivation. This false sterility is obviously due to the frequent failing of dehiscence of anthers whose walls, as referred to before, become conspicuously hardened under the short day condition. The percentage of normal pollen of the varieties as well as that of seed setting are given in Table IV.

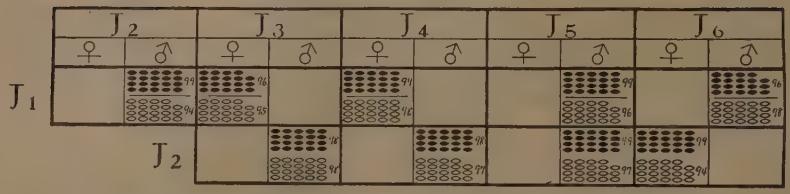
3) *Fertility of the F_1 hybrids*

In the following tables (V–XII) data concerning the percentage of normal pollen and seed setting of the F_1 hybrids originated from 140 matings cultivated under short day teratment are given.

From the first three tables (V–VII) one may conclude that the sexual affinity is complete among the 11 varieties including 6 Japanese, 3 American, and 2 Javanese ones. In all of the hybrids raised among them the pollen is observed to be nearly perfect and their degree of seed setting is comparable to that noted in their parental varieties. In a few cases, however, the degree of seed setting is observed to be little less than 90%. Though these may be ragarded also as completely fertile from the facts that i) the precentage of normal pollen of the hybrids is always more than 90%, ii) the discrepancy between this and the degree of seed setting is comparatively small, and iii) two of their reciprocal matings show more than 90% seed setting. These 11 varieties are considered to be obviously those classified by KATÔ and others as '*Japonica*', which the authors have also grouped and have characterized, together with the other 6 varieties native to Japan Proper (J_7–J_{12}), as Gp. I.

In Table VIII the fertility of the hybrids between the members of Gp. I and five of the varieties of Indian origin is shown, the latter correspond morphologically to those classified by KATÔ and others as '*Indica*' and are confirmed to give rise to completely fertile hybrids *inter se* as shown in Table IX. A striking fact brought out by Table VIII is that the members of Gp. I show considerably high affinity to those Indian varieties. In five of the matings, viz., $J_1 \times I_1$, $J_2 \times I_1$, $NA_1 \times I_1$, $NA_2 \times I_3$, and $I_3 \times SA$, the F_1 hybrids raised are observed to be completely fertile, and in other four, viz., $J_1 \times I_2$, $NA_1 \times I_3$, $J_2 \times I_4$, and $NA_2 \times I_4$, the degree of seed setting of the hybrids is noted always to

TABLE V. Fertility of the F_1 hybrids within Gp. I
1. F_1 hybrids among the varieties native to Japan Proper



● and ○ denote each 5% of normal pollen and seed setting respectively. Small figures in each section show the actual numerical data of the percentage of normal pollen (upper) and seed setting (lower). The same applies to the tables VI–XII

TABLE VI. Fertility of the F_1 hybrids within Gp. I
II. F_1 hybrids among American and Javanese varieties

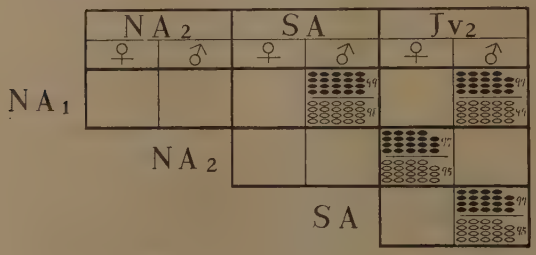
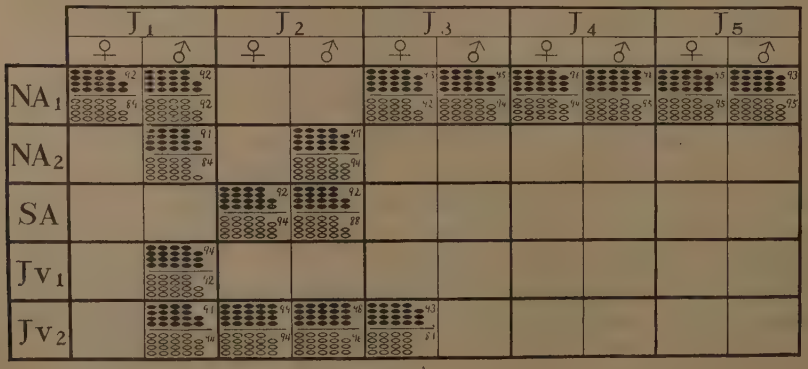


TABLE VII. Fertility of the F_1 hybrids within Gp. I
III. F_1 hybrids between the varieties native to Japan Proper and those of foreign origin



be approximately 90%, although in their pollen cells a slight degree of abortion is recognized. Another noticeable fact is that the hybrids originated from the crosses involving J_3 and I_5 as one parent show remarkable degree of sterility. It is a matter of great interest that these two varieties, forming completely fertile hybrids with their respec-

TABLE VIII. Fertility of the F_1 hybrids Gp. I × Gp. II

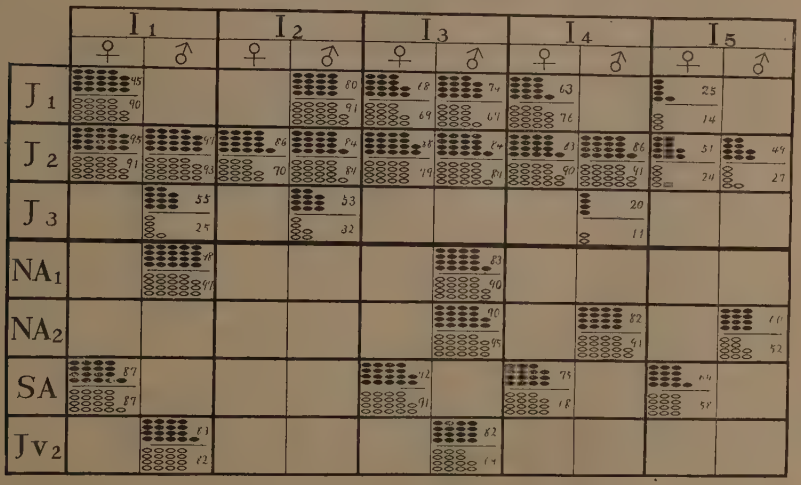
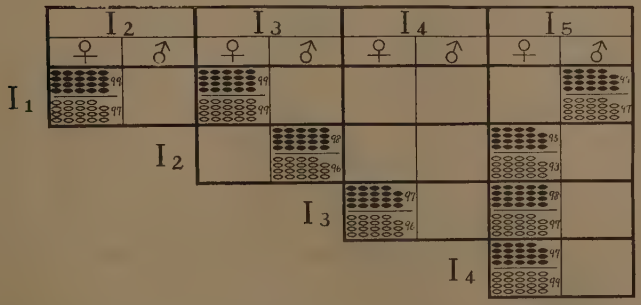


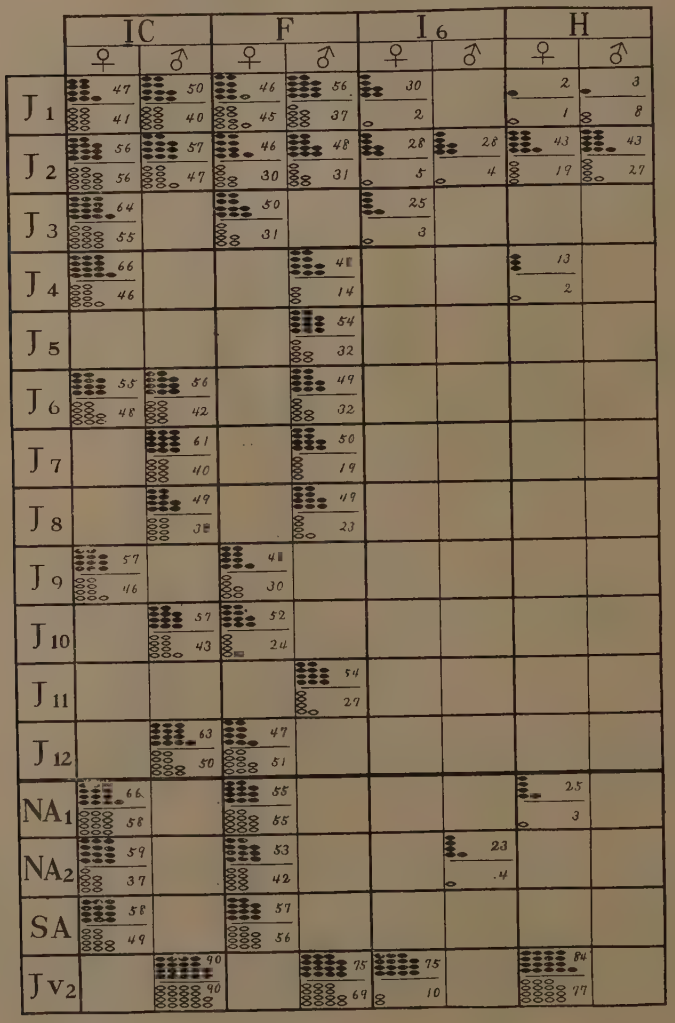
TABLE IX. Fertility of the F_1 hybrids within Gp. II



tive colleagues, display their sexual affinity to the members of each opposite group in quite a different manner. In one of the crosses, $J_3 \times I_4$, there is observed a strong tendency for ovaries to develop parthenocarpically. Here the sterile spikelets contain usually fairly developed ovaries filled up with transparent liquid, which shrivel up soon after the harvest.

Taking aside these, the fertility of the hybrids given rise to in the crosses between the members of Gp. I and the five Indian varieties, which the authors characterize as Gp. II, is markedly high insomuch that these cannot be considered to correspond to the sterile ones reported by KATÔ and others as F_1s *'Japonica'* × *'Indica'*.

TABLE X. Fertility of the F_1 hybrids Gp. I × Gp. III



The results of the crosses made between the members of Gp. I and those of Gp. III, which comprises the remaining four foreign varieties including each one from French Indo-China, Formosa, India, and Hawaii, are shown in Table X. Here the hybrid offspring give considerably high sterility exhibiting the weakest sexual affinity among their parental varieties. As observed, the two varieties, IC and F, give hybrids in the crosses with the members of Gp. I generally with about 50% of pollen abortion and the same degree or more of seed-bearing depression. In those obtained from the matings which involve the two remaining ones, I_6 and H, as one parent, the sterility is utterly high, their percentage of normal pollen being mostly less than 30% and their seed setting being only occasional. It may, however, be mentioned that in these hybrids the degree of abortion in the pollen is not always paralel to that in the embryo-sac, but sometimes there is a striking discrepancy between them. Under microscope about 45% of the embryo-sacs in the hybrids, $H \times J_1$ reciprocally, are found to be normal in spite of the total abortion in the pollen. This is also confirmed by back-crossing them with their parental varieties, in which they give 30–40% seed setting. The fact, in contrast to the case, $H \times J_2$ reciprocally, where the hybrids give about 45% of normal pollen and embryo-sac, suggests the complexity of the mechanism of sexual disharmony among the parental varieties.

Contrary to the authors expectation, a member of Gp. I, Jv_2, presents a conspicuous exception in the sexual affinity to those of Gp. III. The four hybrids having Jv_2 as one parent are highly fertile. One of them, $Jv_2 \times IC$, is considered even to be completely fertile and the other three show more than 75% of normal pollen as well as the corresponding degrees of seed setting, except the one $I_6 \times Jv_2$, which gives a striking seed-bearing depression.

In general the hybrids obtained in the crosses between Gp. I and Gp. III are highly sterile, which are regarded obviously as those reported by KATÔ and others as F_1s *'Japonica'* × *'Indica'*.

In reviewing the fertility of the hybrids originated from the crosses among the members of Gp. III (Table XI), one may notice that in three of the matings, $IC \times I_6$, $H \times F$, and $H \times I_6$, various degrees of sexual disharmony are displayed in quite the same manner as in the cases above described. Also a similar situation previls in the matings between Gp. II and Gp. III (Table XII). Save the F_1s $I_6 \times I_3$ and $H \times I_3$, which show more than 90% in both the normal pollen and the seed setting, the hybrids here obtained cannot be regarded as completely fertile, although, in most cases, they exhibit fairly high degree of fertility. On the other hand, in those having I_5 as one of the parents the degree of sterility is high, which is almost comparable to that noted in the crosses Gp. I × Gp. III (Table X). It is quite interesting that the general situation observed

here closely resembles that observed in those between Gp. I and Gp. II (Table VIII). Further it is of interest that the variety, I_5, which displays remarkably weak affinity to the members of Gp. I also causes high degree of sexual disharmony with those of Gp. III than its colleagues do. Taking aside it, the general aspects suggest strongly that in the sexual affinity the members of Gp. II stand intermediate between those of Gp. I and Gp. III.

TABLE XI. Fertility of the F_1 hybrids within Gp. III

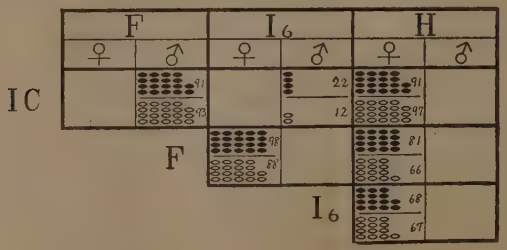
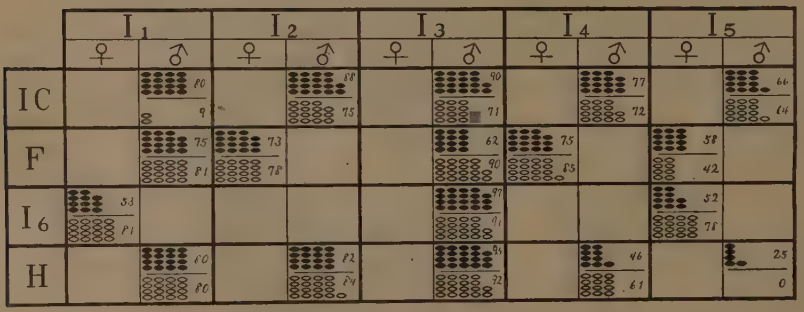


TABLE XII. Fertility of the F_1 hybrids Gp. II × Gp. III



4) *General references to the experimental results*

In reviewing the results of the crossing experiments given in the above tables it may firstly be noticed that the degrees of fertility displayed by the F_1 hybrids are markedly diverse, ranging from more than 80 to almost 0%, except those which should be regarded as completely fertile showing more than 90% in both the normal pollen and the seed setting. KATÔ and others have dealt with 119 series of F_1 hybrids under ordinary paddy field conditions at Hukuoka, Kyûsyû District, of which 67 were raised in the matings made within each of the two groups,

'*Japonica*' and '*Indica*', and 52 between these two. Examination in their fertility by the degree of seed setting has revealed that the hybrids obtained in the crosses within the same group gave 50–90%, mostly 60–80%, whereas those between the different ones usually less than 30%. Some of them were examined as to their degree of pollen abortion, the result of which was that the pollen found in the hybrids within the same group was nearly perfect, while that in those between the different groups was quite imperfect giving only 43–25% normal ones. Now question may arise here as to whether the hybrids with about 80% of normal pollen as well as the corresponding degree of seed setting should be regarded as fertile or, be it ever so slight, partially sterile. As has been described even a pure line which should, no doubt, be completely fertile shows frequently the degree of seed setting far less than 80% in ordinary paddy field culture, though it keeps the percentage of normal pollen always higher than 90% and displays its complete fertility with also more than 90% seed setting if properly treated under favorable conditions. The hybrids in question, however, do not raise usually the percentage both in the pollen and the seed setting under any favorable conditions the authors could have applied and remain as such, except in four cases, $J_1 \times I_2$, $NA_1 \times I_3$, $J_2 \times I_4$, and $NA_2 \times I_4$, (Table VIII), where such hybrids are noted to give approximately 90% seed setting, though their percentage of normal pollen remains always less than 90% indicating a slight degree of abortion. From these facts it may be proper to consider that these hybrids should not be regarded as completely fertile and be distinguished from those whose pollen as well as seed-bearing fertility are comparable to those of the parental varieties.

Another noticeable fact is that in some of the hybrids the percentage of normal pollen is observed to be less than that of seed setting (Table XII). In extreme cases, $I_1 \times I_6$ and $F \times I_3$, the discrepancy between them amount to about 30%. One may recognize that the crosses in which such hybrids are given rise to involve always I_4, F, and I_6 as one parent. Two of the hybrids above-mentioned, $J_2 \times I_4$ and $NA_2 \times I_4$, which give approximately 90% seed setting, but with about 80% of normal pollen, originate also from the matings in which I_4 takes part as one parent. Since in them the degree of pollen abortion remains always constant regardless whether they undergo the short day treatment or not, the surplus pollen sterility in question can be considered to be of purly genetical origin and the three parental varieties to be of peculiar genetical constitution which frequently causes in their hybrid offspring pollen sterility exceeding the degree of embryo-sac abortion.

In each of the hybrids, except those referred to above, the degree of seed setting is observed usually to be less than or, at most, equal to the percentage of normal pollen. The difference between these is noted

to become greater as the dgree of sterility becomes higher. Although there can be considered a certain degree of zygotic lethality, this may be, in greater part, due to the fact that in hybrids of higher sterility anthers, being meagre in contents, fail frequently to dehisce at the time of blooming leaving some embryo-sacs unfertilized. Noteworthy exceptions are, however, given by the two hybrids, $I_6 \times Jv_2$ (Table VIII) and $IC \times I_1$ (Table XII), which present a striking depression in the seed setting despite their high percentage of normal pollen amounting to more than 75%. No explanation for this can be given, as these two hybrids, being obtained from the crosses made in 1937, underwent only one year's investigation and, regret to say, the authors have failed to examine their embryo-sacs.

V. Discussion

The intricate behaviors in the sexual affinity displayed by the 26 varieties used are illustrated by Figure 4. It is recognized certainly that there exists a group of varieties (Gp. III) which correspond to those classified by Katô and others as '*Indica*', forming usually, but by no means laways, sterile hybrids with the varieties also classified by them as '*Japonica*' (Gp. I) which comprises those native to Japan Proper and some others of foreign origin forming completely fertile hybrids *inter se* (see Section I & V in the figure). On the other hand, there is found another group (Gp. II) whose behavior in the sexual affinity cannot so simply be disposed of as that found between the members of the above two groups. In spite of forming completely fertile hybrids among them (Sec. II), the members of this group show their affinity to those of the other in quite variable maners (Sec. IV & VI). I_1 and I_3 give sometimes completely or highly fertile hybrids in crosses with the members of Gp. II and III, but sometimes considerably sterile ones which are comparable to those obtained in the crosses Gp. I × Gp. III. I_2 and I_4 form usually partially sterile hybrids, though most of them are highly fertile including those whose degree of sterility is confined only to a slight abortion in the pollen. I_5, on the contrary, gives rise to always markedly sterile hybrids showing a poor sexual affinity to either of the members of the two groups.

It is noteworthy that among the varieties belonging to so-called '*Indica*' (Gp. III), which after Katô and others, should be considered to have a complete sexual affinity with one another, various degrees of sexual disharmony, although in a few cases, are also displayed (Sec. III). The mode of sterility here observed is considered, in every respects, to be similar to that noted in the sterile hybrids above described, suggesting that underlying mechanism would be common in these cases.

Since only 26 varieties are here used and the number of matings made among them is counted only 140, the data are too small to give a definite conclusion for the interrelationships among the rice varieties. Though it might, at least, be said that the cultivated varieties of rice cannot simply be divided into two distinct groups, such as '*Japonica*' and '*Indica*', by their mutual sexual affinity. Because, the existence of a group of varieties which, from their behaviors, belong neither to

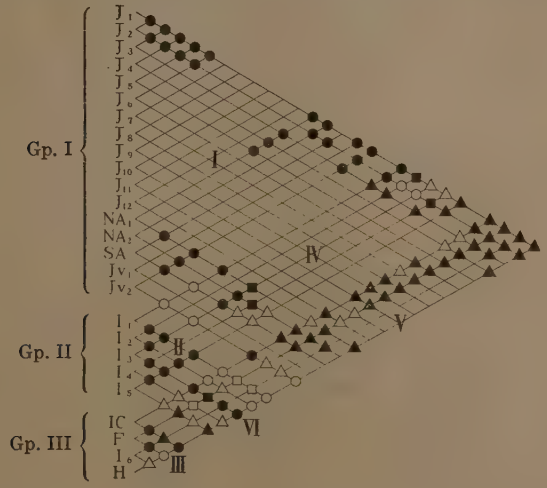


Figure 4. Chart illustrating the mutual sexual affinity among the 26 varieties used

- ●: Completely fertile, the %-age of normal pollen and seed setting of the F_1 hybrids being more than 90%
- ○: Not completely fertile, ...being about 80%
- △: Partially sterile, ...being 80–60%
- ▲: Partially or totally sterile .....being less than 60%
- □: Partially sterile, various discrepancies in the degree of abortion are found between the pollen and the embryo-sac
- ■: Steriality is confined only to the pollen

'*Japonica*' nor to '*Indica*', but stand intermediate between these, renders it difficult to do such a classification.

The gametic sterility found in the F_1 hybrids among the varieties used may not be compared, so far as our results go, with that found by many workers in sterile hybrids seen in the first crosses, except that found by HANEY (unpub. Cf., SANSOME & PHILP, 1932) in *Crepis*. On

crossing two distinctly different forms of *Crepis capilaris*, *C. tectorum*, or *C. rubra*, respectively, he found that the F_1s were only 50% fertile and the F_2s were highly variable. Although no detailed explanation for this was not given, the situation might have partly correspond to that found in our case. As has been described, the degree of gametic abortion displayed by the F_1 hybrids in our case is quite diverse, ranging from 0 to almost 100%, nevertheless the meiosis carried out in them is apparently normal. Further, the segregation of sterility in their selfed progenies is markdly complex as observed in the following examples (Table XIII).

TABLE XIII. Fertility of some F_2 progenies as shown by their percentage of normal pollen

Cross combinations	Percentage of normal pollen in F_1 hybrids	Number of F_2 individuals (The Percentage of Normal Pollen)									Total
		90%	80	70	60	50	40	30	20	10	
$J_1 \times F$	50%	29	29	31	46	61	59	46	21	19	7	348
$F \times J_1$	,,	50	32	41	27	56	41	31	21	8	11	318
$J_1 \times I_3$	70%	127	80	36	31	15	15	2	6	5	0	317

The authors could not attain, for the time being, to a definite conclusion as to the origin of sterility under question, though it might perhaps be said that gene interaction on the one hand and structural rearrangement of chromosomes, such as translocation and segmental interchange, on the other play a part in bringing about it. Owing to the minuteness of chromosomes there can, however, be detected no morphological difference between the two chromosome complements of the parental varieties, which furnishes direct evidence of translocation, nor can be found in the meiosis of sterile hybrids any ring formation, substantiating their interchanged hererozygosity, these can yet be within the range of possibility. PELLEW and SANSOME (1931) have pointed out that, in some cases, gametic abortion accompanying no ring formation can be accounted for on the segmental interchange hypothesis. If the interchange involves only a small part of the chromosomes the frequency of chiasmata in the interchanged parts will be small. Consequently the rings will be rarer than bivalents and the meiosis is apparently normal. Recently MORINAGA (1939), on referring a ring forming semisterile rice plant obtained by him, has also suggested that the individuals with the same degree of sterility, but with apparently normal meiosis, hitherto been reported (ISHIKAWA, 1929; NAKAMURA, 1931; MIYAZAWA, 1932) can be explained as the results of interchanged heterozygosity and the apparent regularity of meiotic division may be due to the minuteness of the interchanged parts not going so far as to cause any ring formation. Granting

here that the sterility involves such chromosome rearrangements, yet the diverse degree of sterility displayed by the F_1 hybrids and its mode of segregation in their F_2 progenies cannot be elucidated by attributing to their effect alone. Hence, there can also be assumed some gene interaction which affectes the viability of gametes.

It is considered that in species of wide geographic and climatic distribution like *O. sativa* L., gene mutation and structural rearrangement may have occurred with the lapse of thousands of years, which may have given rise to so many types that cannot be subjected to a simple classification. The sterility under question may, therefore, be due to the collaboration of these factors, for the clear analysis of which it will take long time and painstaking experiments.

Part II. Relation between the sexual affinity and some morphological characters of the rice varieties

KATÔ and his collaborators have pointed out various morphological differences between *'Japonica'* and *'Indica'* and have concluded that the classification from the morphological stand-point and that from the sexual affinity are in full accord. They have stated that these two groups are distinguished each other by i) the shape and color of the leaf, ii) the angle formed by the uppermost leaf and the stem, iii) the shape of the grain, 4) the presence or absence of the awn, or its amount when present, and v) the length and amount of hairs on the glume. On the other hand HAMADA (1935, '37) has demonstrated that cultivated varieties of rice can be grouped into two types by the organs of sprout, especially the length of mesocotyl (the first internode after BOYD and AVERY), when they are germinated in a dark room at a constant temperature of 30°C. Here he has dealt with more than 300 varieties and has concluded that his classification corresponds fully with that made by KATÔ and his collaborators.

In Part I the authors have described that the interrelationships among rice varieties in view of their mutual sexual affinity are so intricate that they can not simply be divided into two distinct groups. It, therefore, naturally follows that any classification from the morphological view-point, so far as it divides them into two groups, cannot readily be considered to parallel with their sexual affinity. In this part the authors intend to give the results of morphological studies concerning chiefly the form of the paddy or the grain and the length of the mesocotyl, which show obviously the non-parallelism between the sexual affinity and the morphology of rice varieties taken by these workers as differentiating features of the two groups.

I. Materials

Besides the 26 varieties described in Part I the authors have taken 206 ones for their morphological studies, which have also been cultivated at Kônosu Station. These comprise 150 varieties native to Japan Proper and 56 of foreign origin. The latter of these also took parts as parents in our former crossing experiments, though, as their mutual sexual affinity was examined only by the degree of seed setting in F_1 hybrids under ordinary paddy field condition, the exact determination as to whether each of them belongs to Gp. I, II, or III, the authors are not certain. However, from their behaviors observed in more than three matings, 16 of them may be considered as the members of Gp. I and other 10 as those belonging either to Gp. II or III. Their name and origin will be given together with the numerical data in later chapters.

II. Methods

In representing the form of the paddy and the grain the two ratios, length/width and thickness/width, were taken. Of each fully developed paddies taken at random from the respective lots, the three dimensions were measured by means of Shimazu's 'Ames' Dial Guage. Here the rachilla and the awn, if present, were removed carefully from each paddy by the fingers or a small pair of scissors, the former was broken off at the insertion of the outer empty glume and the latter was cut off at the equal height of the top of the palea. After they were investigated the same materials were made into naked grains and were used for further measurements.

In studying the length of mesocotyl the authors have employed the following method which, in some respects, differed from what adopted by Hamada (1931). In culturing rice sprouts Hamada used Sachs' solution with the P_h-value of 6.1-6.7. The authors, however, applied ordinary well-water ($P_h = 6.5–6.7$), because from the results of repeated preliminary experiments they confirmed no noticeable difference in the growth of mesocotyl between the two lots brought up with each different culture solution. As a culture bed a glass dish, being 85 mm. in diameter and 20 mm. in depth, was used, which was covered tightly with a cotton-lace net with 3 × 3 mm. meshes and was filled with water so as to make the net to retain always suitable moisture for the paddies to germinate. On such a bed the germination of paddies is quite uniform and as the sprouts develop their roots come down through the meshes into the water holding the plant body erect. The bed thus prepared was put into a bottomed glass cylinder (diameter, 110 mm.; depth, 130 mm.) with a punched paper lid and was carried into wooden box in a dark room. In order to keep the room temperature to be constantly 30°C., an electric

TABLE XIV. The dimension of the paddy and the grain of the lowland varieties native to Japan Proper
(The figures denote the means of each 50 paddies and grains)

Varieties	Origin	Paddy					Grain				
		Length (L)	Width (W)	Thickness (T)	L/W	T/W	L	W	T	L/W	T/W
		mm.	mm.	mm.			mm.	mm.	mm.		
1. Rikuu 132-gô	Iwate	7.09	3.49	2.43	2.03	0.70	5.05	3.09	2.23	1.63	0.72
2. Iwate Komurasaki Moti 1-gô	,,	6.81	3.30	2.19	2.06	0.66	4.82	2.86	1.98	1.69	0.69
3. Akita 1-gô	Akita	6.84	3.13	2.47	2.19	0.79	4.92	2.66	2.00	1.85	0.75
4. Kameno-o (J_{10})	Yamagata	7.45	3.49	2.40	2.13	0.69	5.37	3.09	2.15	1.74	0.70
5. Igô	,,	7.19	3.31	2.33	2.17	0.70	5.09	2.91	2.15	1.75	0.74
6. Aikoku (J_9)	Hukusima	6.82	3.54	2.40	1.93	0.68	4.93	3.09	2.23	1.60	0.72
7. Ôu 1-gô	,,	7.60	3.42	2.24	2.22	0.65	5.33	3.00	2.02	1.78	0.67
8. Kokuryômiyako (J_{11})	Gunma	7.60	3.55	2.24	2.14	0.63	5.45	3.09	2.24	1.76	0.72
9. Sen'iti (J_3)	Saitama	6.76	3.10	2.19	2.18	0.71	4.83	2.76	2.00	1.75	0.73
10. Omi Sai 5-gô	,,	7.03	3.63	2.07	1.94	0.57	5.26	2.77	1.86	1.90	0.67
11. Husakusirazu Sai 7-gô	,,	7.17	3.43	2.29	2.09	0.67	5.20	3.03	2.13	1.72	0.70
12. Sanzirô Moti Sai 1-gô	,,	6.98	3.41	2.23	2.05	0.65	4.90	2.89	1.99	1.70	0.69
13. Tarobê Moti Sai 1-gô	,,	6.54	3.33	2.19	1.96	0.46	4.58	2.87	1.97	1.60	0.69
14. Murasaki-Ine (J_2)	Kônosu	6.68	3.34	2.24	2.00	0.67	5.05	2.99	2.02	1.69	0.68
15. Sinriki (J_7)	,,	7.77	3.64	2.32	2.13	0.64	5.53	3.17	2.08	1.74	0.66
16. Tyûsei Yamatoriki	Tiba	6.91	3.39	2.18	2.04	0.64	4.98	2.99	1.96	1.67	0.66
17. Kinai Gôriki	,,	7.35	3.61	2.42	2.04	0.67	5.34	3.16	2.22	1.69	0.70
18. Tibanisiki	Tôkyô	7.39	3.69	2.22	3.00	0.60	5.22	3.04	2.00	1.72	0.66
19. Ôzeki	,,	6.89	3.38	2.22	2.04	0.66	4.94	2.96	2.03	1.67	0.69
20. Kamezi (J_4)	Kanagawa	7.35	3.60	2.38	2.04	0.66	5.26	3.15	2.18	1.67	0.69
21. Musasi	,,	6.95	3.42	2.28	2.03	0.67	5.10	3.06	2.06	1.67	0.67
22. Singyoku	,,	7.41	3.45	2.18	2.15	0.63	5.32	3.01	2.03	1.77	0.67
23. Gyokusen	,,	6.96	3.28	2.20	2.12	0.67	5.01	2.90	1.99	1.73	0.69
24. Kairyônisiki	,,	7.23	3.42	2.25	2.11	0.66	5.32	2.98	2.04	1.79	0.68
25. Morita Wase	Niigata	7.19	3.31	2.35	2.17	0.69	5.24	2.91	2.06	1.80	0.71
26. Suitô Nôrin 1-gô	,,	7.02	3.32	2.20	2.11	0.66	4.93	2.91	1.99	1.69	0.68
27. Yonekô	,,	7.33	3.31	2.29	2.21	0.69	5.13	3.06	2.17	1.68	0.71
28. Rikuu 20-gô	,,	6.63	3.53	2.42	1.88	0.69	4.98	3.14	2.30	1.59	0.73
29. Ban 33-gô	,,	7.24	3.59	2.39	2.02	0.67	5.38	3.15	2.19	1.71	0.70
30. Ginbôzu Tyûsei	Toyama	7.58	3.78	2.38	2.01	0.63	4.99	2.87	2.17	1.74	0.76
31. Sin'isiziro	,,	6.85	3.38	2.29	2.03	0.68	5.11	3.01	2.10	1.70	0.70
32. Sirotinko	Hukui	7.21	3.44	2.34	2.10	0.68	5.08	3.05	2.19	1.67	0.72
33. Sihon Moti	,,	7.02	3.28	2.27	2.14	0.69	4.77	2.85	2.04	1.67	0.72
34. Taisyô Moti	,,	7.19	3.54	2.34	2.05	0.67	5.04	2.98	2.14	1.69	0.72

TABLE XIV (*Continued*)

Varieties	Origin	Paddy					Grain				
		L	W	T	L/W	T/W	L	W	T	L/W	T/W
		mm.	mm.	mm.			mm.	mm.	mm.		
35. Sekiyama (J_5)	Yamanasi	6.89	3.58	2.24	1.92	0.63	4.94	3.08	2.05	1.60	0.67
36. Kankisirazu	,,	6.90	3.65	2.38	1.89	0.65	4.95	3.21	2.22	1.54	0.69
37. Hozoroi	,,	6.97	3.45	2.27	2.02	0.66	5.64	3.08	2.05	1.83	0.67
38. Etigo Midumoti	,,	7.31	3.43	2.14	2.13	0.62	5.25	2.91	1.95	1.80	0.67
39. Sekitori (J_6)	Nagano	6.60	3.24	2.23	2.04	0.69	4.87	2.91	2.04	1.67	0.70
40. Sinano Moti 1-gô	,,	7.25	3.49	2.40	2.08	0.69	5.12	3.08	2.19	1.66	0.71
41. Simabôzu 27-gô	Gihu	7.49	3.51	2.38	2.13	0.68	5.26	3.05	2.19	1.72	0.72
42. Wase Sinriki 23-gô	,,	7.41	3.58	2.42	2.06	0.68	5.37	3.20	2.20	1.68	0.69
43. Mino Asahi	,,	7.66	3.48	2.31	2.20	0.66	5.42	3.04	2.07	1.78	0.68
44. Habutae Moti	,,	7.38	3.53	2.26	2.09	0.64	5.02	2.95	2.03	1.70	0.69
45. Miho	Siduoka	7.47	3.52	2.34	2.12	0.66	5.35	3.11	2.16	1.72	0.69
46. Kairyô Akebono	,,	7.25	3.49	2.40	2.08	0.69	5.12	3.08	2.19	1.66	0.71
47. Aiti Kiryôyosi 2-gô	Aiti	7.47	3.67	2.37	2.04	0.65	5.41	3.11	2.12	1.74	0.68
48. Aiti Senbon Asahi	,,	7.51	3.46	2.33	2.17	0.67	5.47	3.08	2.08	1.78	0.68
49. Aiti Mikawanisiki 4-gô	,,	7.44	3.53	2.33	2.19	0.66	5.35	3.14	2.12	1.70	0.68
50. Isenisiki 656-gô	Mie	7.62	3.59	2.23	2.12	0.62	5.39	3.15	2.11	1.71	0.67
51. Siga Watarihune 6-gô	Siga	7.57	3.64	2.40	2.08	0.66	5.47	3.21	2.21	1.70	0.69
52. Yamato Hinode 1-gô	Kyôto	7.66	3.49	2.19	2.19	0.63	5.56	3.05	2.09	1.82	0.69
53. Miyako 1-gô	,,	7.51	3.68	2.36	2.04	0.64	5.64	3.23	2.12	1.75	0.66
54. Katura Moti	,,	7.55	3.24	2.12	2.33	0.65	5.31	2.74	1.89	1.94	0.69
55. Ôsaka Wase Sinriki 8-gô	Osaka	7.37	3.56	2.28	2.07	0.64	5.30	3.13	2.09	1.69	0.67
56. Ôsaka Wase Asahi	,,	7.61	3.52	2.32	2.16	0.66	5.72	3.16	2.16	1.81	0.68
57. Hayakitaho	Hyôgo	7.54	3.55	2.33	2.12	0.66	5.27	3.18	2.07	1.66	0.65
58. Benkei	,,	7.58	3.53	2.40	2.15	0.68	5.58	3.22	2.22	1.68	0.69
59. Asahi	,,	7.59	3.52	2.21	2.16	0.63	5.08	2.93	2.04	1.73	0.70
60. Suitô Nôrin 2-gô	,,	7.39	3.45	2.28	2.14	0.66	5.45	3.00	2.09	1.82	0.70
61. Nagurabo	,,	7.18	3.47	2.27	2.07	0.65	5.61	3.07	2.05	1.83	0.68
62. Hayakitabu 1-gô	Tottori	6.97	3.47	2.40	2.01	0.69	5.06	3.09	2.09	1.64	0.68
63. Hayaôzeki 3-gô	,,	7.24	3.47	2.34	2.09	0.67	5.17	3.09	2.16	1.67	0.70
64. Hattannagare 2-gô	Simane	7.58	3.73	2.39	2.03	0.64	5.43	3.25	2.18	1.67	0.67
65. Gôriki 2-gô	,,	7.39	3.54	2.16	2.09	0.61	5.45	3.07	1.96	1.78	0.64
66. Magatama	,,	7.55	3.49	2.32	2.16	0.66	5.50	2.99	2.07	1.84	0.69
67. Kibiho 2-gô	Okayama	7.50	3.42	2.29	2.19	0.67	5.40	3.04	2.10	1.78	0.69
68. Yamaguti Busaku	Yamaguti	7.43	3.48	2.32	2.14	0.67	5.28	3.11	2.12	1.70	0.68
69. Ôtuti 8-gô	Kagawa	6.97	3.53	2.51	1.97	0.71	4.98	3.14	2.30	1.59	0.73

TABLE XIV (*Continued*)

Varieties	Origin	Paddy					Grain				
		L	W	T	L/W	T/W	L	W	T	L/W	T/W
		mm.	mm.	mm.			mm.	mm.	mm.		
70. Nakate Ôji 73-gô	Kôti	7.76	3.35	2.19	2.32	0.65	5.46	2.93	1.97	1.86	0.67
71. Bansei Koteng 8-gô	,,	7.24	3.51	2.32	2.06	0.66	5.19	3.12	2.10	1.66	0.67
72. Ôbamiyako	Hukuoka	7.44	3.53	2.27	2.11	0.64	5.30	2.99	2.08	1.77	0.70
73. Siratama	,,	7.37	3.57	2.86	2.06	0.66	5.47	3.10	2.18	1.76	0.70
74. Mitui	,,	7.23	3.53	2.30	2.05	0.65	5.17	3.16	2.04	1.64	0.65
75. Omati (J_8)	Saga	7.69	3.64	2.29	2.11	0.63	5.49	3.11	2.05	1.77	0.66
76. Takara	Kumamoto	7.47	3.58	2.35	2.09	0.66	5.52	3.15	2.14	1.75	0.68
77. Sinriki Moti	,,	7.37	3.47	2.26	2.12	0.65	5.11	2.97	2.03	1.72	0.68
78. Yamanaka 2-gô	Miyazaki	7.56	3.53	2.31	2.14	0.65	5.47	3.07	2.09	1.78	0.68
79. Kyûsyû 8-gô	,,	7.44	3.58	2.29	2.08	0.64	5.38	2.98	2.01	1.81	0.67
80. Kônô 35-gô	Kagosima	6.94	3.39	2.26	2.05	0.67	5.01	3.01	2.08	1.66	0.69
81. Tôgô (J_{12})	(?)	7.66	3.30	2.21	2.32	0.67	5.45	2.82	2.01	1.93	0.71

heater was installed in it, whose automatic regulator was put at the equal height of the bottom of the box. From the respective varieties about 100 fully developed and uniform sized padies were taken and these were sown on each different bed. The measurement of the length of mesocotyl was made of about 50 normally developed sprouts after 8 days from the sowing. Of the lots which were to be studied as to the mode of growth of mesocotyl the materials were taken every day continuously from after 3 days until after 8 days from the sowing.

III. EXPERIMENTAL RESULTS

1) *Relation between the sexual affinity and the form of the paddy and the grain*

In demonstrating the morphological difference between '*Japonica*' and '*Indica*' in the form of grain, KATÔ and others have stated that the grain of the former is broad and thick and its cross section is markedly roundish, whereas that of the latter is generally slender and somewhat flat; further, among foreign varieties, belonging to '*Japonica*', some are rarely long, but as compared with those, belonging to '*Indica*', they are broad, and moreover, the transverse section is remarkably roundish.

Firstly, the 26 varieties whose mutual affinity has been confirmed in detail in the crossing experiments described in Part I are considered. The morphology of the paddy as well as the grain found in these varieties is illustrated by the figure 5.

TABLE XV. The dimensions of the paddy and the grain of the upland varieties native to Japan Proper

Varieties	Origin	Puddy					Grain				
		Length (L)	Width (W)	Thickness (T)	L/W	T/W	L	W	T	L/W	T/W
		mm.	mm.	mm.			mm.	mm.	mm.		
1. Suzumesirazu	Aomori	7.94	3.82	2.30	2.08	0.60	5.19	3.12	1.99	1.66	0.64
2. Ryô-on	,,	7.62	3.51	2.14	2.17	0.61	4.92	2.87	1.91	1.71	0.67
3. Tamasari Moti	,,	7.35	3.61	2.20	2.04	0.61	4.93	2.96	1.97	1.67	0.67
4. Kurumi Wase 1-gô	Iwate	7.66	3.51	2.41	2.18	0.69	5.38	3.10	2.02	1.74	0.65
5. Gaisen Moti 13-gô	Miyagi	9.90	3.94	2.50	2.51	0.63	6.58	3.19	2.18	2.06	0.68
6. Akita 1-gô	Akita	8.60	3.58	2.30	2.40	0.64	5.86	2.94	2.02	1.99	0.69
7. Rikuu 22-gô	,,	9.31	3.88	2.47	2.40	0.64	6.30	3.18	2.15	1.98	0.68
8. Mogami Uruti 1-gô	Yamagata	8.62	3.37	2.37	2.56	0.70	5.91	2.97	2.01	1.99	0.68
9. Tikanari 2-gô	,,	7.82	3.28	2.32	2.38	0.71	5.54	2.91	2.00	1.90	0.69
10. Mogami Moti 1-gô	,,	8.33	3.70	2.27	2.25	0.61	5.71	3.03	1.98	1.88	0.65
11. Uruti (Zairai)	Hukusima	7.51	3.45	2.35	2.18	0.68	5.28	2.95	2.02	1.79	0.68
12. Kuroka Moti (J_1)	Ibaragi	7.46	3.76	2·25	1.98	0.60	5.11	3.08	2.02	1.66	0.66
13. Sindaiokosi Ibaragi 1-gô	,,	8.44	3.56	2.27	2.37	0.64	5.76	2.98	1.98	1.93	0.66
14. Kuroka	,,	8.24	3.63	2.53	2.27	0.70	5.72	3.16	2.14	1.81	0.68
15. Rikutô Nôrin 7-gô	,,	7.80	3.67	2.31	2.13	0.63	5.53	3.08	2.04	1.80	0.66
16. Rikutô Nôrin Moti 3-gô	,,	7.91	3.50	2.21	2.26	0.63	5.44	3.11	1.96	1.75	0 63
17. Wase Esozima Moti	Totigi	9.04	3.84	2.42	2.35	0.63	6.19	3.23	2.13	1.92	0.66
18. Nagae Wase Ura 26-gô	Gunma	8.48	3.65	2.37	2.32	0.65	5.93	3.08	2.10	1.93	0.68
19. Yorunoyuki Moti 36-gô	,,	7.97	3.68	2.37	2.17	0.64	5.58	3.03	2.15	1.84	0.71
20. Tôzô Moti b	,,	8.28	3.62	2.34	2.29	0.65	5.84	3.02	2.04	1.93	0.68
21. Urasan	Saitama	8.58	3.70	2.32	2.31	0.63	5.50	3.17	2.05	1.74	0.65
22. Ôhata	,,	8.89	3.94	2.53	2 26	0.64	6.26	3.26	2.25	1.92	0.69
23. Gaisen Moti	,,	9.41	3.86	2.47	2.44	0.64	6.43	3.14	2.15	2.05	0.68
24. Kokkô Moti	,,	8.73	3.67	2.33	2.38	0.63	6.01	2.99	2.04	2.01	0.68
25. Mino Moti Sai 1-gô	,,	8.84	3.54	2.30	2.50	0.65	5.81	2.88	2.02	2.02	0.70
26. Raiden	Kônosu	7.77	3.62	2.19	2.15	0.60	5.58	3.06	1.94	1.82	0.63
27. Hitatinisiki	,,	7.42	3.64	2.31	2.04	0.63	5.34	3.10	2.02	1.72	0.65
28. Yamato	,,	8.02	3.70	2.39	2.17	0.65	5.60	3.12	2.17	1.79	0.70
29. Matuyama	,,	8.18	3.16	2.11	2.59	0.67	6.02	2.70	1.81	2.23	0.67
30. Edogawa	,,	7.46	3.51	2.15	2.13	0.61	5.35	2.95	1.94	1.81	0.66
31. Kôkoku no Homare	,,	7.73	3.69	2.35	2.09	0.64	5.44	3.01	2.09	1.81	0.69
32. Yonaosi	,,	7.89	3.54	2.23	2.23	0.63	5.43	3.01	2.05	1.80	0.68
33. Oiran 1-gô	,,	7.84	3.66	2.22	2.14	0.61	5.47	3.12	1.96	1.75	0.63
34. Yosikawa	,,	7.89	3.43	2.25	2.30	0.66	5.47	2.92	2.01	1.87	0.69
35. Kadusa	,,	7.02	3.65	2.31	1.92	0.63	5.44	3.11	2.05	1.75	0.66

TABLE XV (*Continued*)

Varieries	Origin	Puddy					Grain				
		L	W	T	L/W	T/W	L	W	T	L/W	T/W
		mm.	mm.	mm.			mm.	mm.	mm.		
36. Kônosu Rikutô 1-gô	Kônosu	7.83	3.75	2.35	2.09	0.63	5.62	2.75	2.07	2.04	0.75
37. Kônosu Rikutô 2-gô	,,	8.17	3.93	2.47	2.08	0.63	5.89	3.35	2.17	1.76	0.65
38. Sikoku Moti	,,	8.43	3.77	2.27	2.24	0.60	5.77	3.11	1.99	1.85	0.64
39. Hiderisirasu	Tiba	7.54	3.62	2.19	2 08	0.60	5.32	3.04	1.93	1.75	0.63
40. Tôkyô Hirayama	Tôkyô	8.14	3.77	2.39	2.16	0.63	5.74	3.15	2.15	1.82	0.68
41. Tôkyô Kaneko	,,	8.07	3.78	2.28	2.13	0.60	5.61	3.13	2.04	1.79	0.65
42. Tôkyô Sina Moti	,,	9.39	3.61	2.39	2.60	0.66	6.80	3.00	2.08	2.27	0.69
43. Sensyô	Kanagawa	9.08	4.00	2 52	2.27	0.63	6.33	3.29	2.23	1.92	0.68
44. Owari Moti	,,	9.55	3.77	2.41	2.53	0.64	6.45	3.08	2.10	2.09	0.68
45. Tôzô Moti	,,	8.81	3.51	2.23	2.51	0.64	5.74	2.88	1.96	1.99	0.68
46. Tamasari 1-gô	Toyama	7.42	3.58	2.28	2.07	0.64	5.29	3.02	2.01	1.75	0.67
47. Oiran	Yamanasi	7.30	3.62	2.27	2.02	0.63	5.14	3.02	1.97	1.70	0.65
48. Tôkai 9-gô	Gihu	7.95	3.52	2.19	2.26	0.62	5.44	2.97	1.94	1.83	0.65
49. Yosino Moti 114-gô	,,	9.33	3.54	2.37	2.64	0.67	6.24	2.93	2.05	2.13	0.70
50. Huziokaeri 53-gô	Siduoka	9.15	3.65	2.41	2.51	0.66	6.20	3.06	2.14	2.03	0.70
51. Wase Dango Moti	,,	9.21	3.36	2.42	2.74	0.72	6.15	3.07	2.10	2.00	0.68
52. Kônosuke	,,	8.21	3.28	2 15	2.50	0.66	5.88	2.67	1.90	2.20	0.71
53. Kondô Moti	,,	8.87	3.67	2.42	2.42	0.66	6.09	3.04	2.03	2.00	0.67
54. Aiti Rikutô 2 gô	Aiti	8.12	3.69	2.35	2.20	0.64	5.91	3.15	2.12	1.88	0.67
55. Asaga	Mie	8.75	3.95	2.47	2.22	0 63	6.22	3.27	2.23	1.90	0.68
56. Rikutô Nôrin Moti 1-gô	,,	8.46	3.69	2.39	2.29	0.65	5.85	3.10	2.12	1.89	0.68
57. Tôkai 15-gô	,,	7 50	3.58	2.30	2.09	0.64	5.31	3.00	2.06	1.77	0.69
58. Tôkai 10-gô	,,	7.86	3.79	2.37	2.07	0.63	5.64	3.19	2.11	1.77	0.66
59. Tôkai 1-gô	,,	8 31	3.74	2.30	2.22	0.61	5.98	3.15	2.22	1.90	0.70
60. Tôkai 2-gô	,,	8.18	3.79	2.24	2.16	0.59	5.65	3.16	1.94	1.79	0.61
61. Tôkai 11-gô	,,	7.96	3.75	2.41	2.12	0.64	5.90	3.19	2.15	1.85	0.67
62. Ôhata Wase	,,	8.05	3.69	2.33	2.18	0.63	5.83	3.22	2.12	2.81	0.66
63. Tôkai 5 gô	,,	8.43	3.83	2.35	2.20	0.61	5.85	3.18	2.04	2.84	0.64
64. Dango Moti	Kyôto	8.92	3.66	2.23	2.44	0.61	6.16	3.05	1.98	2.02	0.65
65. Mino Moti	Hyôgo	8.39	3.67	2 31	2.29	0.63	5.80	3.03	2.05	1.91	0.68
66. Rikutô Nôrin 5-gô	Tottori	8.47	3.71	2.38	2.28	0.64	5.89	3.16	2.08	1.86	0.66
67. Hino Wase	,,	9.00	3.70	2.41	2.43	0.65	6.14	3.02	2.09	2.03	0.69
68. Yamahata Wase	Ehime	9.53	3.68	2.36	2.59	0.64	6.73	3.12	2.11	2.16	0.68
69. Kuma Moti	,,	9.18	3.55	2.26	2.59	0.64	6.19	2.95	2.03	2.10	0 69
70. Tazima 1-gô	Kumamoto	8.63	3.43	2.06	2.52	0.60	5.98	2.89	1.89	2.07	0.65
71. Kirisima	,,	7.73	3.67	2.25	2.11	0.61	5.67	3.06	2.00	1.85	0.65
72. Sensyôho	,,	8.80	3.92	2.50	2.24	0.64	6.40	3.27	2.24	1.96	0.69

TABLE XV (*Continued*)

Varieties	Origin	Puddy					Grain				
		L	W	T	L/W	T/W	L	W	T	L/W	T/W
		mm	mm.	mm.			mm.	mm.	mm.		
73. Kumamoto 1-gô	Kumamoto	9.31	3.65	2.40	2.55	0.66	6.31	3.01	2.11	2.10	0.70
74. Rikutô Sinriki 1-gô	Miyazaki	8.23	3.50	2.06	2.35	0.59	5.72	2.93	1.81	1.95	0.62
75. Satuma 5-gô	Kagosima	8.24	3.50	2.17	2.35	0.62	6.91	2.90	1.88	2.38	0.65
76. Satuma 2-gô	„	8.21	3.42	2.16	2.40	0.63	5.64	2.98	1.91	1.89	0.64
77. Hakaburi 1-gô	„	8.42	3.93	2.49	2.14	0.63	5.90	3.26	2.23	1.81	0.68
78. Kahei	„	8.85	3.71	2.32	2.39	0.63	6.81	3.14	2.57	2.17	0.82
79. Gaisen Moti 1-gô	„	9.42	3.69	2.35	2.55	0.64	6.27	3.00	2.21	2.09	0.74
80. Rikutô Nôrin Moti 6-gô	„	8.28	3.55	2.25	2.33	0.63	5.57	2.89	2.00	1.93	0.69
81. Hakaburi 20-gô	„	7.61	3.49	2.31	2.18	0.66	5.44	2.94	2.06	1.85	0.70

It is recognized that the ratio, length/width (= L/W), found in the varieties native to Japan Proper ($J_1 \ldots J_{12}$) is always below 2.5 in the paddy and below 2.0 in the grain, whereas that found in those of foreign origin, including the members of Gp. I, II, and III, is, in most cases, above 2.5 and above 2.0 respectively. In the ratio, thickness/width (= T/W), however, no noteworthy difference is noted among them. In 24 cases out of 26, it falls between 0.6 and 0.8, leaving the two of the members of Gp. II, I_3 and I_5, above it.

In the form of paddy it seems as if these varieties were divided into two groups in regard to their mutual affinity. Because, taking aside the two, NA_2 and SA, all the members of Gp. I are found below the limit, L/W = 2.5, and those of Gp. II and III, lacking, more or less, the affinity to the former, above it. In the form of grain, however, they take quite a random distribution, save the varieties native to Japan Proper which arrange themselves always below 2.0. The general situation observed only indicates that the varieties of foreign origin have usually longer grains than those native to Japan Proper, irrespective of their affinity to the latter. Further, it cannot be said that the transverse section of the grain of the varieties classified by KATÔ and others as *'Japonica'* (Gp. I) is more roundish than that of those classified by them as *'Indica'* (Gp. II and III). On the contrary, the most roundish one is found in such varieties, I_3 and I_5, whose grains are most slender, showing the characteristics typical of *'Indica'*. It is interesting that the morphology of paddy and grain observed in the members of Gp. III resembles closely that observed in those of Gp. I, i.e., the similarity in morphology is rather recognizable between the two remotest groups than between those (Gp. I

TABLE XVI. The dimension of the paddy and the grain of the varieties of foreign origin

Varieties	Origin	Paddy					Grain				
		Length (L)	Width (W)	Thick-ness (T)	L/W	T/W	L	W	T	L/W	T/W
		mm.	mm.	mm.			mm.	mm.	mm.		
1. Dai-Sei-Mô	Manchuria	7.10	3.50	2.32	2.03	0.65	4.96	2.92	2.05	1.70	0.70
2. Gyoku-Haku-Kô	Korea	8.01	3.61	2.15	2.22	0.60	5.62	3.07	1.87	1.83	0.61
3. Haku-Koku-Sô	Formosa	8.01	3.31	2.19	2.42	0.66	5.65	2.86	1.97	1.98	0.69
4. Ukoku-Sô	,,	8.00	3.21	2.16	2.49	0.67	5.67	2.71	1.91	2.09	0.70
5. Oka-Ine* (F)	,,	8.80	3.00	1.95	2.61	0.64	6.16	2.56	1.84	2.41	0.72
6. Haku-Koku-Nen	China (Zyûkei)	8.38	2.51	1.96	2.34	0.78	6.13	2.19	1.74	2.80	0.79
7. Tô-Teki-Sai	,, ,,	8.86	3.09	2.02	2.87	0.65	6.24	2.58	1.77	2.42	0.69
8. Sui-Haku-Zyô	,, (Seito)	8.43	2.98	2.04	2.83	0.68	5.98	2.55	1.81	2.35	0.71
9. Ti-Tô	,, (?)	8.46	3.14	2.12	2.69	0.68	5.85	2.63	1.89	2.22	0.72
10. Kô-Hoku-Tô	,, (Kôhoku)	8.57	2.55	2.96	3.36	0.77	6.28	2.20	1.71	2.85	0.78
11. Ban-Sen*	,, (Shanghai)	8.13	3.20	2.20	2.54	0.69	5.81	2.77	1.96	2.10	0.71
12. Tyô-Ko-Tô	,, (Sansei)	14.10	2.95	2.41	4.78	0.82	9.48	2.50	1.20	3.79	0.50
13. Sai-Si-Yô-Sen*	,, (Kôso)	8.62	2.60	1.93	3.32	0.74	6.22	2.55	1.74	2.44	0.68
14. Ro-Sen	,, (Sekkô)	8.48	2.91	1.97	2.91	0.68	6.11	2.43	1.75	2.51	0.72
15. Kô-Sen*	,, ,,	8.82	2.81	1.99	3.14	0.71	6.23	2.28	1.73	2.73	0.76
16. U-Koku	,, ,,	8.78	3.12	2.03	2.81	0.65	6.26	2.53	1.75	2.47	0.69
17. Kwan-Non-Sen*	,, ,,	8.27	2.87	1.96	2.88	0.68	6.48	2.57	1.73	2.52	0.67
18. Sin-Ha-Sen*	,, (Kôsyû)	9.04	2.98	2.15	3.03	0.72	6.54	2.58	1.88	2.53	0.73
19. Gin-Nen	,, (Kwang-tung)	7.95	2.60	1.93	3.06	0.74	5.82	2.18	1.68	2.67	0.77
20. Hyaku-Zitu-Sô°	,, (Nangking)	—	—	—	—	—	5.48	2.56	1.76	2.14	0.69
21. Kwaku-Ittyô°	,, ,,	—	—	—	—	—	5.77	2.64	1.97	2.18	0.75
22. Surjamkhi* (I_1)	India (Calcutta)	8.56	2.60	1.87	3.29	0.72	5.82	2.26	1.66	2.58	0.73
23. Charnack* (I_5)	,, ,,	9.19	2.17	1.73	4.24	0 80	6.81	1.83	1.56	3.72	0.85
24. Mushakdanti* (I_3)	,, (Bombay)	8.74	2.01	1.62	4.35	0.81	6.11	1.73	1.44	3.53	0.83
25. Modan	,, ,,	9.57	3.15	2.23	3.04	0.71	6.79	2.70	2.02	2.51	0.75
26. Bason Takakal* (I_2)	,, (Assam)	8.07	2.60	1.86	3.10	0.72	5.94	2.28	1.66	2.61	0.73
27. Baurash Murai	,, ,,	8.63	1.95	1.62	4.43	0.83	6.26	1.71	1.44	3.66	0.84
28. Lepedumai	,, ,,	8.36	2.77	1.95	3.02	0.70	5.91	2.39	1.74	2.47	0.73
29. Kangni	,, (Sind)	9.30	2.78	1.97	3.35	0.71	6.69	2.41	1.78	2.78	0.74
30. Jajai	,, ,,	9.94	2.64	1,90	3.77	0.72	6.92	2.84	1.78	2.44	0.63
31. Sonahiri	,, ,,	10.54	2.73	1.98	3.86	0.73	7.31	2.38	1.83	3.07	0.77
32. Red Kangro*	,, ,,	10.31	2.63	1.94	3.92	0.74	7.29	2.28	1.78	3.20	0.78
33. Karalath* (I_4)	,, (?)	6.19	2.47	1.71	2.51	0.69	4.52	2.17	1.51	2.08	0.70
34. Danahara* (I_6)	,, ,,	8.00	3.06	1.95	2.61	0.64	5.52	2.64	1.74	2.09	0.66
35. Hatadavi*	,, ,,	9.39	2.98	2.03	3.15	0.68	6.79	2.53	1.80	2.68	0.71

TABLE XVI (*Continued*)

Varieties	Origin	Paddy					Grain				
		L	W	T	L/W	T/W	L	W	T	L/W	T/W
		mm.	mm	mm.			mm.	mm.	mm.		
36. Te-tep* (IC)	Indo-China (Hanoi)	7.81	2.70	1 85	2.89	0.69	5.84	2.33	1.65	2.51	0.71
37. Chiêm Chanh	,, ,,	—	—	—	—	—	6.22	2.45	1.80	2.53	0.73
38. Lua Rong*	,, ,,	8.39	2.56	1.87	3.28	0.73	6.10	2.20	1.67	2.77	0.76
39. Nep Vai	,, ,,	8.80	3.25	2.13	2.71	0.66	6.31	2.74	1.85	2.30	0,68
40. Ketan Nangka° (Jv_2)	Java	8.21	3.46	2.32	2.37	0.67	5.56	2.83	2.11	1.96	0.75
41. Ketan eson°	,,	9.34	3.58	2.24	2.61	0.63	6 61	2.84	1.99	2.33	0.70
42. Loktjan° (Jv_1)	,,	8.35	3.55	2.22	2.35	0.63	6.34	3.02	2.00	2.10	0.66
43. Padi Poeti Boeloe	,,	8.96	3.17	2.28	2.83	0.72	6.76	2.73	2.15	2.48	0.79
44. Hawaii no. 154* (H)	Hawaii	7.40	2.93	2.04	2.56	0.70	5.13	2.58	1.75	1.99	0.68
45. Hawaii no. 17*	,,	9.78	3.13	2.20	3.12	0.70	7.35	2.70	1.88	2 72	0.70
46. Tadukan	Philippine (Davao)	8.16	2.59	1.83	3.15	0.71	5.94	2.36	1.63	2.52	0.70
47. Basilanon	,, ,,	9.61	2.48	1.48	3.88	0.60	7.24	2.10	1.67	3.45	0.80
48. Louisiana Awnless°	U. S. A.	8.01	3.02	2.09	2.65	0.69	5.93	2.55	1.82	2.33	0.71
49. Carolina° (NA_1)	,,	8.07	3.30	1.98	2.45	0.60	6.18	2.77	1.76	2.23	0.64
50. Hondulas° (NA_2)	,,	9.63	3.26	2.07	2.95	0.63	7.46	2.80	1.82	2.66	0.65
51. Daddy Wrigh°	,,	10.00	3.30	2 20	3.03	0.67	7.51	2.28	1.97	3.29	0.86
52. Vintura°	,,	9.84	3.27	2.18	3.01	0.67	7.41	2.65	1.91	2.80	0.72
53. Texas Fortuna°	,,	10.14	2.30	2.00	4.41	0.87	7.55	2.56	1.78	2.95	0.70
54. Fortuna°	,,	10.09	3.01	2.03	3.35	0.67	7.63	2.49	1.78	3.06	0.71
55. Jaguary° (SA) (glutinous)	South America (Saõ Paulo)	10.21	3.48	2.17	2.93	0.62	7.27	2.89	2.04	2.52	0.71
56. Jaguary° (nonglutinous)	,, ,,	—	—	—	—	—	6.55	2.66	2.02	2.46	0.75
57. Ferrao Preto°	,, ,,	8.63	3.10	2.13	2.78	0.69	6.42	2.67	1.88	2.40	0.70
58. Jamaica°	,, (Lima)	10.12	3.17	2.12	3.19	0.67	7.50	2.66	1.86	2.82	0.70
59. Carolina Dorado°	,, ,,	9.53	3.11	2.12	3.06	0.68	7.13	2.64	1.89	2.70	0 72
60. Tambo°	,, (Arequipa)	10.30	3.28	2.14	3.14	0.65	7.50	2.76	1 90	2.72	0.69
61. Amareria°	,, (?)	9.05	3.13	1.97	2.89	0.63	6.81	2.50	1.86	2.72	0.74
62. Amareriyo°	,, ,,	9.91	3.21	2.13	3.09	0.66	7.27	2.64	1.85	2.75	0.70
63. Katete°	,, ,,	8.65	3.40	2.21	2.54	0.65	6.39	2.92	1.99	2.19	0.68
64. Higasi-Africa-san-Akamai	Eastern Africa	9.46	3.30	2.13	2.87	0.65	6.97	2.66	1.83	2.62	0.69

TABLE XVI (*Continued*)

Varieties	Origin	Paddy					Grain				
		L	W	T	L/W	T/W	L	W	T	L/W	T/W
		mm.	mm.	mm.			mm.	mm.	mm.		
65. Sogleng	(?)	8.12	3.50	2.19	2.32	0.63	5.96	2.91	1.96	2.05	0.67
66. Koneng	,,	9.05	3.41	2.41	2.65	0.71	6.43	3.02	2.16	3.13	0.72
67. Djidah	,,	9.39	3.19	2.08	2.94	0.65	6.86	2.65	1.86	2.59	0.70
68. Boerajoet	,,	9.47	3.53	2.22	2.68	0.63	7.10	2.85	1.94	2.49	0.68
69. Rantaj-emas 2	,,	9.31	3.53	2.38	2.64	0.67	7.20	3.07	2.12	2.35	0.69
70. Carolina Saigon	,,	9.32	2.93	2.10	3.18	0.72	6.82	2.49	1.80	2.74	0.72

°: varieties which belong or are supposed to belong to Gp. I.
*: varieties which belong or are supposed to belong either to Gp. II or Gp. III.

and II) which are comparatively closely related in view of the sexual affinity.

Quite the same situation as above can be observed in the relation between the sexual affinity and the morphology of paddy and grain among the whole varieties investigated as shown in Fig. 7. Here it is again recognized that there exists generally a fair distinction in the slenderness, L/W, between the varieties native to Japan Proper and those of foreign origin, but no noticeable difference in the form of its transverse section. Further, it is observed that the slenderness of the paddy or of the grain is more or less independent of the sexual affinity among these varieties. Hence, it can be concluded that the form, more particularly the slenderness, of paddy or grain distinguishes the varieties of foreign origin from those native to Japan Proper, but by no means determines the sexual affinity among them.

2) *Relation between the length of mesocotyl and the sexual affinity*

In classifying the rice varieties into two groups by the length of mesocotyl, HAMADA has based upon the following criterion that those below and above 10 mm. are '*Japonica*' and '*Indica*' respectively. These two groups are also distinguished from each other, as he states, by the ratio between the length of mesocotyl and that of the coleoptile. In '*Japonica*' it is always below 40%, while in '*Indica*' always above it.

Before giving the explanation concerning the relation between the sexual affinity and the length of mesocotyl among the varieties used, it should be mentioned that the experiments, which were repeated thrice, were carried out each at different temperature. Owing to the hitch of the automatic switch attached to the temperature regulator, the temperature in the dark room indicated by the recording thermometer was

in the first experiment 26–28°C in the second 30°C constant, and in the third 30–33°C.

The length of mesocotyl measured at 8 days after the sowing was observed to be considerably different in each variety at these three

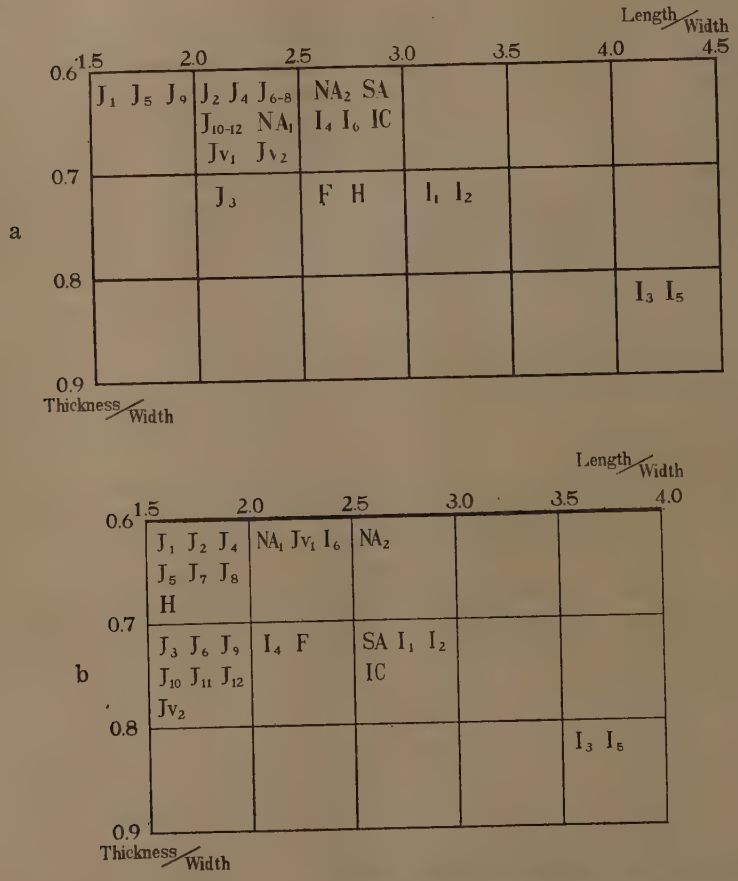


Figure 5. Form of the paddy (a) and the grain (b) found in the three groups classified in the crossing experiments

Gp. I: J_1....J_{12} (varieties native to Japan Proper), NA_1, NA_2, SA, Jv_1, Jv_2
Gp. II: I_1....I_5
Gr. III: IC, F, I_6, H

different grades of temperature, as observed in the examples shown in Table XVII. As is observed, the length of mesocotyl usually, but by no means always, becomes longer as the temperature becomes higher near

30°C. At 26–28°C only five of the varieties of Indian origin, forming Gp. II (I_1....I_5), show their length of mesocotyl above 10 mm., leaving all the remaining ones below it. At 30°C const., however, a disparity in length

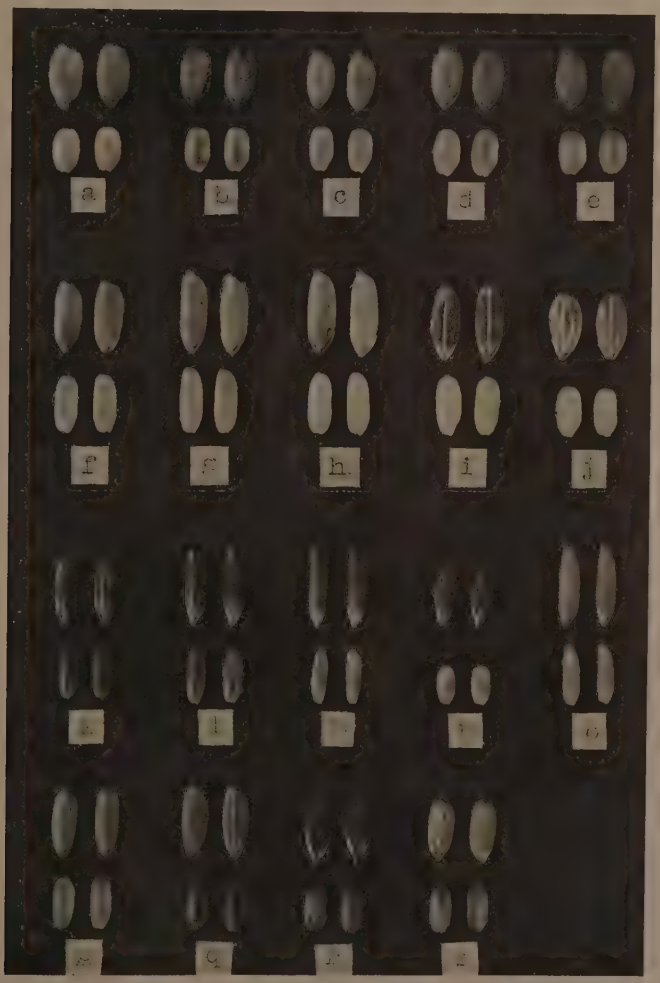


Figure 6. Photogragh showing the morphology of paddy and grain of the varieties of the three groups

a–j: Gp. I. a, J_1. b, J_2. c, J_3. d, J_4. e, J_5. (varieties native to Japan Proper) f, NA_1. g, NA_2. h, SA. i, Jv_1. j, Jv_2
k–o: Gp. II. k, I_1. l, I_2. m, I_3. n, I_4. o, I_5
p–s: Gp. III. p, IC. q, F r, I_6. s, H

among them becomes fairly distinct. In some of them it increases rather slowly remaning still under 10 mm. or exceeding only a few mm. above it, while in others it elongates quite rapidly reaching above 30 to above

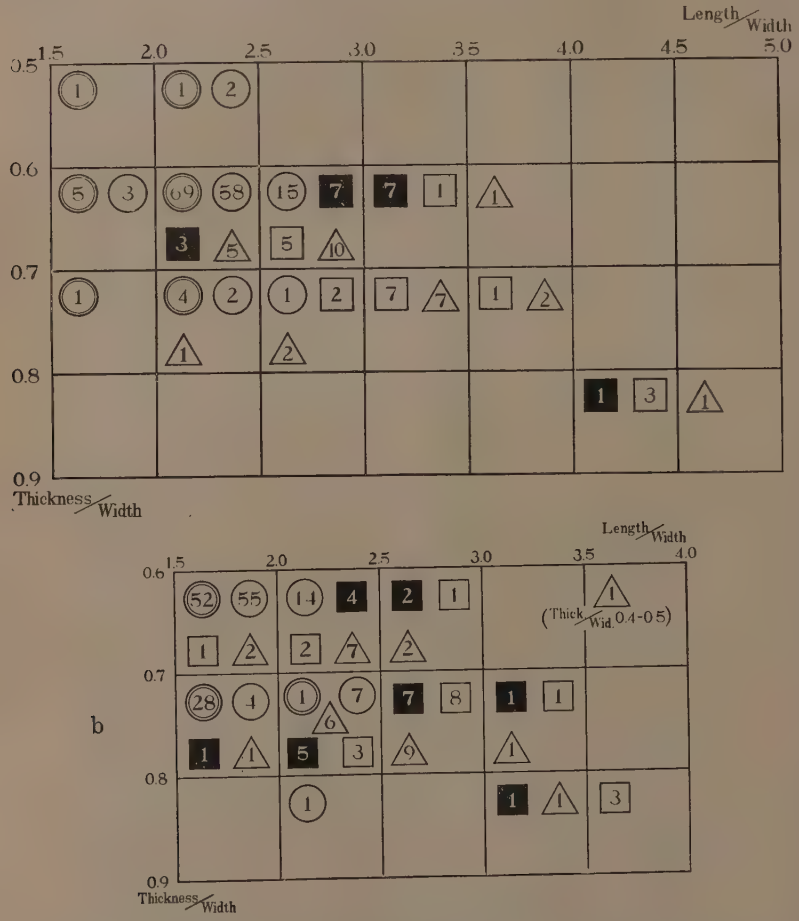


Figure 7. Relation between the sexual affinity and the form of paddy (a) and Grain (b) among the 232 varieties investigated

◎: Lowland varieties native to Japan Proper
○: Upland varieties native to Japan Proper
■: Foreign varieties which belong or are supposed to belong to Gp. I
□: Foreign varieties which belong or are supposed to belong either to Gp. II or to III, lacking, more or less, the affinity to those of Gp. I
△: Foreign varieties whose sexual affinity is not certain
The figures in the signs denote the number of varieties

70 mm. At 30–33°C a considerable difference can be observed even among those which remained below 10 mm. at 30°C const. Though, a few varieties, I_3 and I_6, show the maximum length at 30°C const. undergoing a sudden depression at 30–33°C and in another, IC, it tends to decrease as the temperature rises. In such varieties it is considered that the rise of temperature induces the rapid growth of the other organs of sprout,

TABLE XVII. Relation between the temperature and the growth of mesocotyl and coleoptile

	Temperature / Varieties	26–28°C			30°C const.			30–33°C		
		Length of Mesocotyl (M)	Length of Coleoptile (C)	M/C %	M	C	M/C %	M	C	M/C %
		mm.	mm.		mm.	mm.		mm.	mm.	
Gp. I	J_1 , Kuroka Moti	4.7	32.1	14.6	7.5	35.0	21.4	16.3	38.1	42.8
	J_2 , Murasaki Ine	4.2	32.4	12.9	8.1	41.0	19.7	16.1	35.2	45.7
	J_3 , Sen'iti	3.4	27.2	12.5	7.2	34.0	21.2	11.3	32.1	35.2
	J_4 , Kamezi	3.0	30.9	9.7	4.5	32.4	13.8	6.5	34.2	19.0
	J_5 , Sekiyama	6.2	35.2	17.6	6.0	39.7	15.1	9.3	39.0	23.8
	J_6 , Sekitori	5.1	37.5	13.6	6.6	38.2	17.2	8.1	34.4	23.5
	NA_1, Carolina	7.7	26.8	28.7	30.0	37.0	81.0	40.3	35.6	113.2
	NA_2, Honduras	7.9	30.0	26.3	11.8	31.0	38.0	16.6	32.1	51.7
	SA, Jaguary	7.5	28.8	26.0	13.3	35.8	37.1	14.1	33.9	41.6
	Jv_1, Loktjan	5.8	27.2	21.3	14.9	36.0	41.3	26.0	26.6	97.7
	Jv_2, Ketan Nangka	9.1	31.0	29.3	14.2	36.0	39.4	15.8	31.2	50.6
Gp. II	I_1 , Surjamkhi	18.3	24.2	75.6	31.0	26.2	118.3	30.6	22.8	134.2
	I_2 , Bason Takakal	16.4	21.9	74.8	41.0	28.8	142.3	52.7	26.1	201.9
	I_3 , Mushakdanti	24.1	25.4	94.8	72.9	19.7*	370.0	48.6	23.9	203.3
	I_4 , Karalath	21.2	29.5	71.8	49.5	26.8	184.7	90.2	21.6	417.5
	I_5 , Charnack	13.9	31.0	44.8	33.8	33.8	100.0	96.6	32.8	294.5
Gp. III	IC , Te-tep	2.7	28.0	9.6	1.8	31.8	5.6	0.7	26.3	2.6
	F , Oka-Ine	6.8	38.5	17.6	9.3	39.0	23.8	9.2	32.2	28.5
	I_6 , Danahara	6.8	28.3	24.0	75.2	12.8*	587.5	64.7	16.9*	322.8
	H , Hawaii no. 154	4.3	31.1	13.8	6.9	35.9	19.2	6.9	26.2	26.3

* The sudden depression in length of coleoptile is due to that it has not reached to its maximum growth even after 8 days from the sowing

viz., coleoptile, the first, and the second leaves at the comparative earlier stage of mesocotyl development, resulting in the depression of growth of the latter.

The variation in length of coleoptile noted at these three different grades of temperature is rather small compared with that of mesocotyl.

From 26 to 28°C, it generally becomes longer as the latter increases its length. From 30°C const. to 30–33°C, however, it does not go with the mesocotyl, tending usually to decrease as the latter increases. Since its maximum length is, in most cases, found at 30°C const., the value of the ratio, M/C (= length of mesocotyl/length of coleoptile), at 30–33°C is consequently larger than that at 30°C const. Two of the varieties native to Japan Proper, J_1 and J_2, and three of the foreign ones belonging to Gp. I, NA_2, SA, and Jv_2, which give the ratio under 40% at 30°C const., exceed it by about 2–12% at 30–33°C. Hence, the varieties showing their length of mesocotyl to be 10–20 mm. and the ratio, M/C, to be near 50%

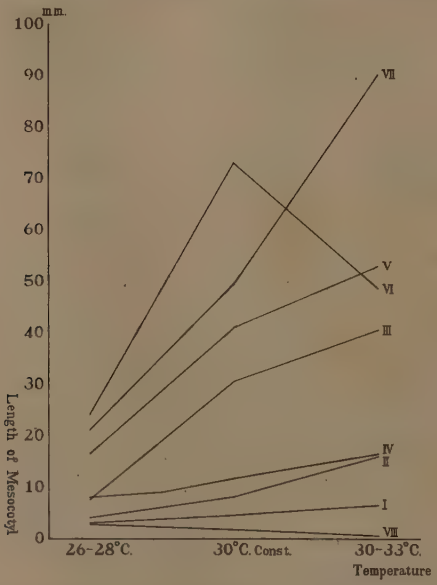


Figure 8. Relation between the temperature and the length of mesocotyl
I, J_4. II, J_2. III, NA_1. IV, NA_2. V, I_2. VI, I_3. VII, I_4. VIII, IC

at 30–33°C, may be taken approximately as those, of which the former is below or little more than 10 mm. and the latter is below 40% at 30°C const.

Firstly the length and the mode of growth of mesocotyl at 30°C const. observed in the members of the three groups may be closely scrutinized. One may recognize that the mode of growth and the length of mesocotyl presented by the six varieties native to Japan Proper (J_1.... J_6), correspond fully to those of '*Japonica*' reported by HAMADA. In these varieties the mesocotyl reaches its stage of maximum growth

comparatively early and its length is always below 10 mm. (Fig. 9, a.). Further, the ratio, M/C, found in them is, with no exceptions, below 40% (Table XVII). On the other hand, the five varieties of foreign origin, NA_1, NA_2, SA, Jv_1, and Jv_2, belonging also to Gp. I, and show their length of mesocotyl above 10 mm. Though, its mode of growth resembles fairly that observed in the varieties native to Japan Proper and the ratio, M/C, is only a little below or above 40%, except the one, NA_1, which

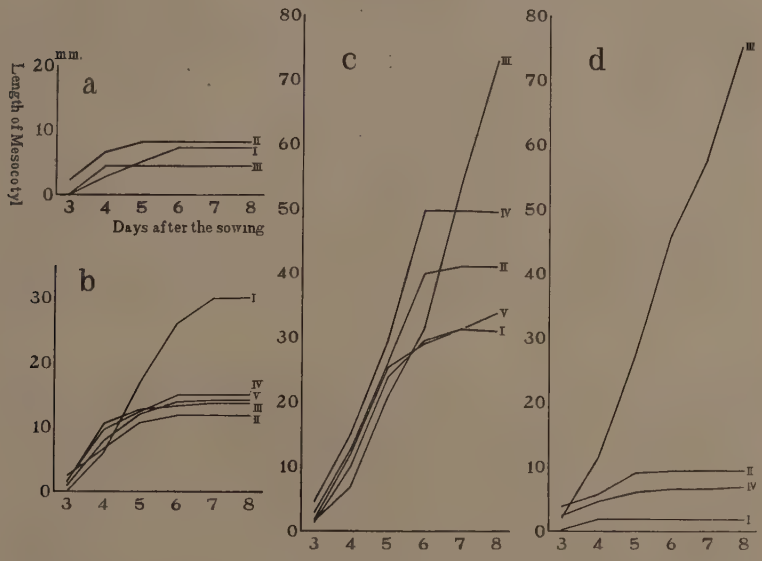


Figure 9. The mode of growth of mesocotyl found in the varieties of the three groups.

a: Gp. I. I, J_1. II, J_2. III, J_4. (varieties native to Japan Proper)
b: Gp. I. I, NA_1. II, NA_2. III, SA. IV, Jv_1. V, Jv_2. (varieties of foreign origin)
c: Gp. II. I, I_1. II, I_2. III, I_3. IV, I_4. V, I_5
d: Gp. III. I, IC. II, F. III, I_6. IV, H

is, in every respects, considered to be '*Indica*' after HAMADA (Fig. 9, b). In the five varieties of Indian origin ($I_1 \ldots I_5$), forming Gp. II, one can see those of typical '*Indica*' (Fig. 9 c.). Here the growth curve is markedly steep. It reaches its maximum value far later after the sowing than do the varieties classified by him as '*Japonica*' and, in some cases, it still goes upwards even after 8 days. The length of mesocotyl and the ratio, M/C, given by these varieties exceed far beyond the limits, 10 mm. and 40%, respectively, which divide the two groups,

'Japonica' and *'Indica'*. It is noteworthy that the members of Gp. III which are characterized to have the weakest sexual affinity to those of Gp. I, cannot be distinguished from those native to Japan Proper in

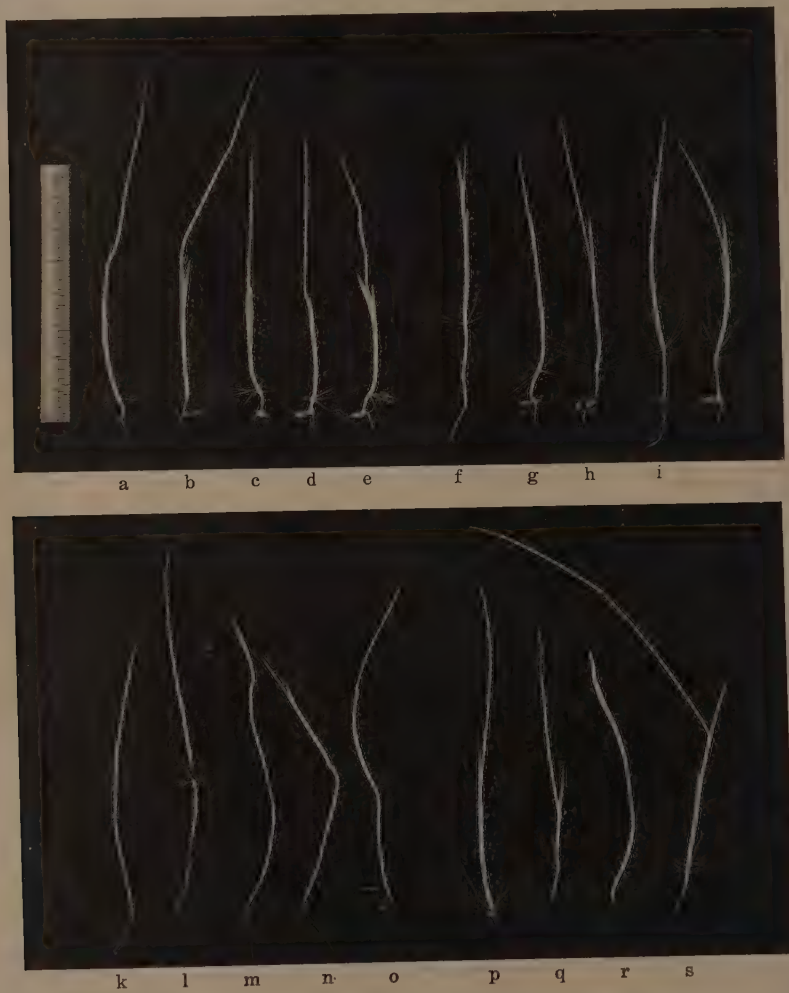


Figure 10. Photographs showing the length of mesocotyl in each of the varieties of the three groups

a–j: Gp. I. a, J_1. b, J_2. c, J_3. d, J_4. e, J_5. (varieties native to Japan Proper). f, NA_1. g, NA_2. h, SA. i, Jv_1. j, Jv_2
k–o: Gp. II. k, I_1. l, I_2. m, I_3. n, I_4. o, I_5
p–s: Gp. III. p, IC. q, F. r, I_6. s, H

their length as well as their mode of growth of mesocotyl, save the variety of Indian origin, I_6, which presents quite the same behavior as that observed in the members of Gp. II (Fig. 9, d.) The length and the ratio, M/C, observed in them are below 10 mm. and 40% respectively and the growth curve is quite similar to that presented by the varieties native to Japan Proper.

From the facts above described one may find, as far as these materials are concerned, an obvious discrepancy between the classification made by the sexual affinity and that by the length of mesocotyl, even granting here that the four varieties of Gp. I (NA_2, SA, Jv_1, & Jv_2), whose length of mesocotyl is above 10 mm., but the ratio, M/C, is

TABLE XVIII. The length of mesocotyl and coleoptile of the lowland varieties Native to Japan Proper. (at 30–33°C)

Var. no.	The length of Mesocotyl (M)	The length of Coleoptile (C)	M/C %	Var. no.	M	C	M/C %	Var. no.	M	C	M/C %
1	3.8	31.7	12.0	28	2.2	36.3	6.1	55	6.7	35.9	18.7
2	5.8	26.8	21.6	29	3.6	29.7	12.1	56	11.0	31.2	35.3
3	6.4	39.9	16.0	30	2.8	32.3	8.7	57	15.5	30.2	51.3
4	3.2	28.0	11.4	31	2.4	31.4	7.6	58	8.8	30.7	28.7
5	6.6	31.9	20.7	32	3.8	27.6	13.8	59	4.1	25.6	16.0
6	1.5	38.3	3.9	33	5.7	32.9	17.3	60	4.4	30.4	14.5
7	6.9	30.6	22.5	34	6.5	39.3	16.5	61	2.0	30.1	6.6
8	7.5	30.7	24.4	35	9.3	39.0	23.8	62	2.1	26.1	8.0
9	13.7	31.6	43.4	36	17.8	31.3	56.9	63	3.6	41.8	8.6
10	5.1	32.6	15.6	37	4.1	33.8	12.1	64	13.7	31.6	43.4
11	4.4	24.3	18.1	38	3.4	31.8	10.7	65	11.2	28.3	39.6
12	5.9	27.8	21.2	39	8.1	34.4	23.5	66	3.0	27.5	10.9
13	7.3	34.6	21.1	40	5.9	32.1	18.4	67	7.5	26.7	28.1
14	16.1	35.2	45.7	41	3.8	37.3	10.2	68	7.8	25.1	31.1
15	6.3	34.4	18.3	42	3.5	26.2	13.4	69	3.8	34.1	11.1
16	1.8	31.5	5.7	43	5.1	28.5	17.9	70	2.3	30.1	7.6
17	3.3	40.8	8.1	44	3.3	43.0	7.7	71	8.0	26.8	29.9
18	3.1	34.8	8.9	45	3.5	37.1	9.4	72	3.0	21.9	13.7
19	2.0	25.6	7.8	46	5.4	29.8	18.1	73	12.0	26.2	45.8
20	6.5	34.2	19.0	47	4.2	27.9	15.1	74	1.8	27.0	6.7
21	2.9	32.8	8.8	48	4.9	30.8	15.9	75	7.8	25.3	30.8
22	3.8	28.7	13.2	49	7.8	23.7	32.9	76	2.6	25.7	10.1
23	2.8	29.8	9.4	50	4.7	28.7	16.4	77	3.4	22.5	15.1
24	7.5	30.1	24.9	51	9.4	27.4	34.3	78	8.2	28.7	28.6
25	9.6	43.8	21.9	52	9.9	25.6	38.7	79	3.4	25.2	13.5
26	5.2	33.8	15.4	53	9.2	26.7	34.5	80	2.7	27.2	9.9
27	11.8	27.1	43.5	54	5.6	37.6	14.9	81	3.3	38.1	8.7

approximately 40%, to be *'Japonica'*. Because neither do the crosses made among the varieties of different categories in view of the length of mesocotyl always result in the formation of sterile F_1 hybrids, nor those among the varieties of the same category always in the formation of fertile ones.

TABLE XIX. The length of mesocotyl and coleoptile of the upland varieties native to Japan Proper. (at 30–33°C)

Var. no.	The length of Mesocotyl (M)	The length of Coleoptile (C)	M/C %	Var. no.	M	C	M/C %	Var. no.	M	C	M/C %
1	21.4	26.3	34.2	28	8.2	33.7	24 3	55	4.5	24.9	18.1
2	30.2	30.5	99.0	29	22.6	27.7	81.6	56	9.1	25.5	35.7
3	24.3	37.3	65.1	30	22.7	34.4	66.0	57	85.9	34.4	249.7
4	27.5	35.0	78.5	31	4.7	39.1	12.0	58	18.8	28.8	65.3
5	12.0	31.0	38.7	32	26.1	36.7	71.1	59	18.7	27.6	67.8
6	15.3	32.9	46.5	33	29 8	39.8	74.9	60	46.1	35.1	131.3
7	16.0	35.3	45.3	34	18.4	36.8	50.0	61	8.2	27.2	30.1
8	40.3	42.2	47.6	35	33.5	34.7	96.5	62	6.8	26.3	25.9
9	6.2	22.3	27.8	36	21.2	36.8	57.6	63	10.0	27.6	36.2
10	16.4	36.0	45.5	37	16.2	32.0	50.6	64	20.2	31.1	65.0
11	14.2	25.5	55.7	38	26.3	33.5	78.5	65	9.9	32.7	30.3
12	16.3	38.1	42.8	39	19.7	25.7	76.7	66	15.8	29.4	53.7
13	19.3	33.6	57.4	40	10.0	31.5	31.7	67	10.6	30.7	34.5
14	8.9	24.4	36.4	41	17.5	28.9	60.6	68	10.3	32.1	32.0
15	28.2	34.4	82.0	42	16.8	34.3	49.0	69	15.9	33.9	46.9
16	11.1	31.4	35.5	43	3.8	24.4	15.6	70	13.4	26.8	50.0
17	13.3	32 9	40.0	44	15.6	36.5	42.6	71	13.4	25.0	53.6
18	31.2	25.8	120.9	45	9.1	28.0	32.5	72	5.4	27.1	19.9
19	18.8	34.0	55.3	46	21.0	31.6	66.5	73	17.4	31.4	55.4
20	7.2	28.4	25.4	47	23.5	33.2	70.8	74	11.7	30.0	39.0
21	18.6	34.0	54.7	48	26.7	37.5	71.2	75	13.9	28.0	49.6
22	45.5	23.8	19.1	49	12.1	33.7	35.9	76	16.2	26.4	61.4
23	12.2	35.6	34.2	50	5.6	22.2	25.2	77	30.4	32.7	93.0
24	9.1	29.2	31.2	51	18.7	33.5	55.8	78	15.0	27.3	54.9
25	8.0	25.1	31.9	52	22.4	33.9	66.1	79	14.3	29.0	49.3
26	10.9	24.3	44.9	53	9.1	26.7	34.1	80	16.3	33.4	51.5
27	35.1	34.1	103.2	54	11.6	26.8	43.3	81	27.0	31.0	87.1

It is noticed here that the members of Gp. II stand in remarkable contrast to those of Gp. I in the mesocotyl length and its mode of growth. On the other hand, it is also noticeable that the members of Gp. III behave in quite the same manner as those of Gp. I, except I_6. A morphological resemblance is again manifested, likely in the case with the

TABLE XX. The length of mesocotyl and coleoptile of the varieties of foreign origin (at 30–33°)

Var. No.	Origin	The length of Mesocotyl (M)	The length of Col optile (C)	M/C %	Var. No.	Origin	M	C	M/C %
1	Manchuria	18.9	37.2	50.8	36*	Indo-China	0.7	26.3	2.7
2	Korea	18.3	44.1	41.5	37	,,	2.3	21.7	10.6
3	Formosa	79.5	27.5	289.1	38*	,,	1.9	24.2	7.9
4	,,	88.4	46.1	191.8	39	,,	3.0	22.6	1.3
5*	,,	9.2	32.2	28.6	40°	Java	15.8	31.2	50.6
6	China	20.7	33.4	62.0	41°	,,	7 2	27.0	26.7
7	,,	9.3	24.8	37.5	42°	,,	26.0	26.6	96.7
8	,,	16.3	25.6	63.7	43	,,	16.3	32.9	49.5
9	,,	17.5	43.2	40.5	44*	Hawaii	6.9	26.2	26.3
10	,,	—	—		45*	,,	14.6	36.9	39.6
11*	,,	17.4	37.9	45.9	46	Philippine	0.3	24.2	1.2
12	,,	13.9	41.9	33.2	47	,,	15.3	23.5	65.1
13*	,,	27.6	36.9	74.8	48°	U.S.A.	12.2	37.2	32.8
14	,,	10.8	26.7	40.4	49°	,,	40.3	35.6	113.2
15*	,,	18.2	32.8	55.5	50°	,,	16.6	32.1	51.7
16	,,	16.1	38.2	42.1	51°	,,	25.6	35.1	72.9
17*	,,	11.7	40.2	29.1	52°	,,	4.9	23.4	20.9
18*	,,	4.1	40.7	10.1	53°	,,	12.6	32.5	38.8
19	,,	17.2	37.3	46.1	54°	,,	21.3	30.4	70.1
20°	,,	35.0	36.0	97.2	55°	South America	14.1	33.9	41.6
21°	,,	3.8	25.3	15,0	56°	,,	8.4	26.5	31.7
22*	India	30.6	22.8	134.2	57°	,,	10.6	28.1	37.7
23*	,,	48.6	32.8	148.2	58°	,,	7.2	26.5	27.2
24*	,,	96.6	23.9	404.2	59°	,,	6.8	23.4	29.1
25	,,	32.1	27.9	115.1	60°	,,	18.4	25.2	73.0
26*	,,	52.7	26.1	201.9	61°	,,	10.7	25.5	42.0
27*	,,	85.7	20.4	420.1	62°	,,	9.1	29.4	31.0
28	,,	71.3	31.0	230.0	63°	,,	9.5	30.9	30.7
29	,,	40.3	30.2	133.4	64	Eastern Africa	2.3	16.4	1.4
30	,,	138.0	19.0	726.3	65	?	13.0	27.1	48.0
31	,,	63.4	33.1	191.5	66	,,	10.1	27.8	36.3
32*	,,	107.7	37.6	286.4	67	,,	13.8	36.3	38.0
33*	,,	90.2	21.6	417.6	68	,,	12.4	31.0	40.0
34*	,,	64.7	16.9	382.8	69	,,	11.2	30.7	36.5
35*	,,	39.0	22.6	172.6	70	,,	8.7	27.7	31.4

° : varieties which belong or are supposed to belong to Gp. I.
* : varieties which belong or are supposed to belong either to Gp. II or Gp. III.

form of paddy and grain, between two sexually remoter groups than between those which are comparatively closely related.

In considering the situation with the whole materials it may be noticed that all the samples examined were taken from the lots which were cultured at 30–33°C. Since the mesocotyl is confirmed to tend, in most cases, to increase in length as the temperature rises near 30°C, the data here given may not be scrutinized appropriately by the criterion of 10 mm. as in the case of 30°C const. Nevertheless, they are observed to be sufficient to establish the facts above described.

TABLE XXI. Relation between the sexual affinity and the length of mesocotyl among the whole varieties investigated

		(Length of mesocotyl in mm.) 0	10	20	30	40	50	60	70	80	90	100	Total
Lowland varieties native to Japan Proper		72	9										81
Upland varieties native to Japan Proper		18	38	16	5	3				1			81
Varieties of Foreign Origin	Those belong or are supposed to belong to Gp. I	8	8	3	1	1							21
	Those belong or are supposed to belong either to Gp. II or III	5	4	1	2	1	1	1		1	2	1	19
	Those whose sexual affinity is not certain	5	15	1	2	1		1	2	1		1	29

In reviewing Table XXI, in which one can see the relation between the length of mesocotyl and the sexual affinity among the whole varieties investigated, it may firstly be noticed that there is a remarkable contrast in the length of mesocotyl between the lowland and upland ones native to Japan Proper. The overwhelming part of the former is below 10 mm. even at the comparatively high temperature of 30–33°C, whereas in the latter it ranges from above 10 mm. to above 40 mm., leaving 18 below 10 mm. and 1 above 80 mm. HAMADA (1938), in his study with the upland varieties cultivated in Japan Proper, has stated that, from their length of mesocotyl, about 30% of them should be regarded as '*Indica*', which are supposed to be introduced in Japan comparatively recently as inferred from literatures published in the 17th and the 18th century. As some of the varieties, showing their length of mesocotyl below 10 mm. at 30°C const., are found to give 10–20 mm. at 30–33°C, so if we take here those above 20 mm. to be '*Indica*' of HAMADA, about 30% of the investigated upland varieties are regarded as such, corresponding to his view. Though, from the results of many a crosses undertaken in our breeding procedure at Kônosu Station among the upland varieties

themselves as well as between them and the lowland ones, it cannot obviously be accepted that the difference in mesocotyl length concerns the sexual affinity among them. As an example illustrative of that, a strain, Tôkai No. 15, can be mentioned, which originated from the cross between two upland varieties, Hakaburi × Tamasari No. 1, and was bred at Mie Prefectural Agricultural Experiment Station. In spite of the comparatively short mesocotyls of the parents (20–30 mm. at 30–33°C), the strain shows its length above 80 mm., which is comparable to that found in the members of Gp. II, though it causes no sexual disharmony in crosses with other upland varieties.

Secondly, one may notice that among the varieties of foreign origin, having full sexual affinity to those native to Japan Proper, there are some which show their length of mesocotyl above 20 mm., and that among those, which belong or are supposed to belong either to Gp. II or III, showing more or less weak affinity, some are found to have their length even below 10 mm.

Judging from the facts above described, the classification of rice varieties into two groups according to their length of mesocotyl made by HAMADA, cannot be considered to parallel the mutual sexual affinity among them, although it is of importance owing to another reason.

IV. DISCUSSION

The author have described here the obvious non-parallelism between the sexual affinity and two of the morphological characteristics which have been considered by some workers to be parallel to each other. Further, it has been recognized that the two morphological extremities (Gp. I and Gp. II) in regard to the form of paddy or grain and the length of mesocotyl by no mean display the poorest sexual affinity. On the contrary, the highest degree of sexual disharmony is manifested between such groups (Gp. I and Gp. III) which are morphological rather related. These two characters, therefore, may probably be due to some genic differences which are more or less independent of the ones responsible for the sexual disharmony. One of them, the length of mesocotyl, is transferable from one type to another without concerning the sterility, which has been referred to elsewhere (MIDUSIMA and YAMADA, 1939).

It, is, however, noteworthy that the varieties native to Japan Proper and those native to India stand morphologically in extreme contrast to each other, although the differentiating features seem, in most cases, to have little or no relation with the sexual affinity between them. The form of paddy or grain found in the former is quite thickset, while that found in the latter is markedly slender (Table XIV, XV, and XVI).

The length of mesocotyl at 30–33°C observed in the former is mostly under 20 mm., whereas in the latter it shows always more than 30 to 100 mm. Furthermore, they exhibit remarkable difference in some physiological traits. In a diffused light (600–1,000 Lux), for instance, most of the varieties of Indian origin show a remarkable feebleness in the chlorophyl formation, while in those native to Japan Proper it is observed to be of far slighter degree (MIDUSIMA, unpublished). From these facts it is considered appropriately that they should be distinguished from each other as the remotest groups in view of the morphological as well as the physiological make-up, although the discrimination is not readily applicable for characterizing their mutual affinity.

In the foregoing part it has been suggested that the intricate mode of gametic sterility displayed by the F_1 hybrids among the rice varieties might have been broguht about by the collaboration of genic changes and structural rearrangements of chromosomes produced in them during past thousands of years in their wide climatic and geographic distribution. It may again be considered that these are also responsible for changes in their external morphology and physiological make-up. On the other hand, there might have occurred a number of other genic changes independent of the mutual sexual affinity among them. In addition, being a crop of great importance, they might have been subjected to an extreme artificial selection during these long times, which will contribute to the isolation of various forms with subsequent independent mutation. These, cooperating together, might have given rise to various extreme types reaching even the specific rank. Hence, it cannot so simply be considered, similarly as in the case with their mutual sexual affinity, that they are classified into two distinct groups. Consequently, any classification from the morphological view-point hitherto been made, so far as it divides them into two groups separated in view of the sexual affinity, canot readily be accepted.

In closing, the authors wish to express their sincere thanks to H. KÔNO, K. SAITÔ, T. YAMADA, T. HIROSE, S. ENDÔ and Y. YASUDA for much technical assistance during the course of the experiments.

Summary

1. The results of crossing experiments comprising 140 combinations made among 26 rice varieties which include 12 ones native to Japan Proper and 14 ones of foreign origin are described.

2. F_1 hybrids obtained in these crosses show diverse degrees of gametic sterility, ranging from 0 to almost 100% as shown by their degree of pollen abortion.

3. The microsporogenesis carried out in the sterile hybrids proceeds apparently normally. Nevertheless, degeneration of microspores takes place in various degree before their maturation. The macrosporogenesis is considered to follow usually a similar course, giving, in most cases, the degree of embryo-sac abortion corresponding to that of the pollen.

4. The sexual disharmony is recognized not only between the varieties native to Japan Proper and nine of those of foreign origin, but also among the latter themselves. The mode of sterility observed in both cases is considered, in every respects, to be similar, the underlying mechanism of which would be expected to be common.

5. The 26 varieties used may be classified, at least, into three groups by their degree of mutual sexual affinity. Group I comprises twelve Japanese and five foreign varieties (including 2 North American, 1 South American, and 2 Javanese ones), forming completely fertile hybrids *inter se*. Gp. III consists of four foreign varieties (including each one native to French Indo-China, Formosa, India, and Hawaii) which give usually highly sterile hybrids in crosses with those of Gp. I. Gp. II contains five varieties of Indian origin occupying an intermediate position between the above two, which sometimes give rise to completely or highly fertile hybrids, but in other cases utterly sterile ones in crosses with the respective members of Gp. I and Gp. III.

6. From these facts it is concluded that the classification of rice varieties into two distinct groups, '*Japonica*' and '*Indica*', by their mutual sexual affinity made by KATÔ and his collaborators should be in needs of a reconsideration.

7. The difference in the morphology of paddy and grain, which is considered by the above workers as one of the differentiating features of the two groups, has been examined on 232 varieties originating from chief rice-producing regions in the world. Also the difference in the length of the mesocotyl which is employed by HAMADA as the criterion in discriminating the two groups from the morphological view-point, has been investigated on the same material.

8. The results show that these morphological differences cannot be considered to be parallel to the sexual affinity among the varieties. Furthermore, the similarity in morphology is rather recognizable between the two remotest groups (Gp. I and Gp. III) in view of the sexual affinity than between those (Gp. I and Gp. II) having comparatively high affinity to each other.

9. It is concluded that any classification from the morphological view-point, so far as it divides the rice varieties into two distinct groups sexually separated, cannot readily be accepted.

Literature

BOYD, L. and AVERY, G. S. Jr. 1936. Grass-seedling anatomy: the first internode of *Avena* and *Triticum*. Bot. Gaz. **79**; 765–779.

HAMADA, H. 1931. Über die Beeinflussung des Wachstums des Mesokotyls und der Koleoptile von *Avena*-Keimlingen. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. **6**; 161–238.

———. 1933. Wachstumsverhältnisse der Keimorgane von verschiedenen Gramineen in Dunkel und bei Belichtung mit besondere Berücksichtigung ihrer systematischen Stellung. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. **9**; 71–128.

———. 1935. Nippon Rikutô ni Niti In Ryôkei ari (On the occurence of '*Japonica*' and '*Indica*' types among the upland rice varieties native to Japan Proper.) Agr. and Hortic. **10**; 2530–2532.

———. 1937. Physiologisch-systematische Untersuchungen über das Wachstum der Keimorgane von *Oryza sativa* L. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. **12**; 259–309.

———. 1938. Gaseikikwan no Seityô ni yoru Java-Tô no Bunrui (Classification of rice varieties native to Java by different modes of growth of the sprout organs). Commemoration papers on agronomy prepared in honour of Prof. M. AKEMINE. Yôkendô, Tokyo; 69–73.

———. and MAZIMA, M. 1938. Nippon Suiriku Ryôtô no Sakusô ni tuite (On the complication of lowland and upland rice varieties in Japan). Comm. Pap. Agro. prep. in hon. of Prof. M. AKEMINE. Yôkendô, Tokyô; 74–84.

HIRAYOSHI, I. 1937. Species hybrids between cultivated rice and wild rice (*Oryza latifolia*) with special reference to their sterility and maturation division (A preliminary note). Jap. Jour. Gen. **8**; 59–60.

ISHIKAWA, J. 1937. Studies on the inheritance of sterility in rice. Jour. Coll. Agr. Hokkaido Imp. Univ. **20**; 79–201.

——— 1931. Ruirei naki Idengensyô no Kenkyû (Studies on genetical phenomena without analogies). Agr. and Horti. **6**; 227–230.

JONES, J. W. 1930. Sterility in rice hybrids. Jour. Am. Soc. Agr. **22**; 861–867.

KATÔ, S. 1930. On the affinity of the cultivated varieties of rice plants, *Oryza sativa* L. Jour. Dept. Agr. Kyûsyû Imp. Univ. **2**; 241–276.

———., KOSAKA, H., and HARA, S. 1928. On the affinity of rice varieties as shown by the fertility of hybrid plants. Bult. Sci. Fakult. Terkult. Kyûsyû Imp. Univ. **3**; 132–147.

———., MARUYAMA, Y. 1928. Serodiagnostic investigation on the affinities of different varieties of rice. Bult. Sci. Fakult. Terkult. Kyûsyû Imp. Univ. **3**; 132–147.

MIDUSIMA, U. and YAMADA, Y. 1939. Some considerations on the relation between F_1-sterility and mesocotyl length among Japanese and foreign rice varieties, *O. sativa* L. Jap. Jour. Gen. **15**; 14–18.

MIYAZAWA, B. 1932. On the two cases of semi-sterility in *O. sativa* L. Bull. Miyazaki Coll. Agr. and Forest. **4**; 193–197.

MORINAGA, T. 1939. Ine no Saibô-Idengaku (Cyto-genetics in *Oryza*). Bot. and Zool. **7**; 197–201.

NAKAMURA, S. 1931. Cytological observations in two forms of sterile rice plant. Proc. Crop Sci. Soc. Japan. **7**; 259–265.

PELLEW, C. and SANSOME, E. R. 1932. Genetical and cytological studies on the relations between Asiatic and European varieties of *Pisum sativum*. Jour. Gen. **25**; 25–54.

SANSOME, F. W. and PHILP, J. 1932. Recent advances in plant genetics. Churchill, London.

Critical observations on the origin of the blepharoplast and centrosome in plants

By Akira YUASA

(Received August 22, 1939)

I. Introduction

Though various opinions have been advanced concerning the origin of the blepharoplast in the plant kingdom, it is now generally recognized that the blepharoplast and centrosome belong to one and the same double structure which acts as a centrosome in some cases and as a blepharoplast in other cases (cf. FUJII 1930). But even if this conclusion is admitted the origin of the blepharoplast(1) still remains undetermined, for the very reason that the origin of the double structure in question has not yet been traced.

Summarizing the results obtained by the various authors, IKENO (1906) classified the blepharoplast into three groups, namely: 1) "zentrosomatische Blepharoplasten", 2) "plasmodermale Blepharoplasten", 3) "Karyo- oder Kernblepharoplasten". According to M. WILSON (1911) the blepharoplasts may be classified into four categories, namely 1) the blepharoplast which appears *de novo* in the cytoplasm, 2) the blepharoplast which occurs as the dense portion of the cytoplasm (this corresponds to the "plasmodermale Blepharoplasten" of IKENO, 3) the blepharoplast which is homologous with the centrosome and is derived from the latter (this corresponds to the "zentrosomatische Blepharoplasten" of IKENO, 4) the blepharoplast of nuclear origin (this corresponds to the "Kernblepharoplasten" of IKENO. YAZAWA (1931) classified the blepharoplast into four categories, namely : 1) the blepharoplast which is derived from the centrosome (centrosomal blepharoplast), 2) the blepharoplast which occurs *de novo* in the cytoplasm (cytoplasmic or endoplasmic blepharoplast), 3) the blepharoplast which is differentiated from the plasma membrane (plasmodermal blepharoplast), 4) the blepharoplast derived from the nucleolus (nucleolar blepharoplast). According to LANG (1936) it may be classified into three categories, namely : 1) the blepharoplast

(1) In this paper, the term "blepharoplast" means the body which has not yet been differentiated into border-brim, cilia-bearing band and lateral bar. In the cases of bacteria, Myxomycetes and Flagellatae this limitation is unnecessary.

which is thought to be the organ *sui generis*, 2) the blepharoplast composed of chondriosome-substance, 3) the blepharoplast derived from the nucleolus. However, all these considerations can be included in the following two categories, namely : 1) the blepharoplast is derived from the nucleus, 2) the blepharoplast is derived from the cytoplasm or cytosomes.

It is recognized by WILSON (1928) and SHARP (1934) that the blepharoplast and centrosome belong to one and the same structure: the direct evidence in favor of this opinion being the cases of *Ochromonas granularis* by DOFLEIN (1918), *Stemonitis flaccida* by JAHN (1904) and *Stemonitis splendens* var. *flaccida* and *St. flavogenita* by the present writer (1936, 1937), *Cycas revoluta* by IKENO (1898), *Marchantia polymorpha* by IKENO (1903), *Equisetum arvense* by SHARP (1912) and *Marsilia quadrifolia* by SHARP (1914). On the base of his studies the present writer believes the double structure theory of the blepharoplast and centrosome to be correct.

In this paper, the origin of the blepharoplast[1] is first discussed and then that of the centrosome is considered. Most of the statements are based on direct observations of the present writer, though some points are only suppositional.

II. Materials and Methods

As materials the following species of plants were used: *Fuligo septica*, *Stemonitis flavogenita*, *St. splendens* var. *flaccida*, *Neurospora crassa*, *Peziza* sp., *Pyronema* sp., *Dictyota dichotoma*, *Marchantia polymorpha*, *Adiantum capillus-veneris*, *Dryopteris oligophlebia* var. *elegans*, *Pteris cretica* var. *albo-lineata*, *Equisetum arvense*, *Allium Cepa*, *Vicia Faba*, *Plantago lanceolata*. These materials are observed in tap water, artificial sea water or 1–2% saccharose solution. For purposes of comparison some materials were also fixed, sectioned according to the paraffin method and stained with HEIDENHAIN's iron-alum haematoxylin. The aceto-carmine method and FEULGEN's nucleal staining method were also used in the present study.

III. The origin of the blepharoplast

1. THE BLEPHAROPLAST DERIVED FROM THE NUCLEUS

In Pteridophyta the present writer (1937, 1938) had already recognized from the following facts that the blepharoplast is derived from the nucleus.

(1) The origin of blepharoplast was once discussed in the BOTANICAL MAGAZINE (Tokyo) Vol. **52** (1938): 318–328 by the present writer.

1) Generally the spherical blepharoplast appears, at first, in the spermatid in Eufilicinean plants. [In *Nephrodium molle,* however, YAMANOUCHI (1908) observed the blepharoplast in the spermatid mother cell.] The centrosome cannot be observed in the spermatogenous mitoses.(1) Accordingly the first occurrence of the blepharoplast is in the spermatid in Eufilicinean plants, with the single exception of *Nephrodium.*

---

(1) In the spermatogenous division of Eufilicinean plants the centrosome cannot be observed but the blepharoplast can be observed in the spermatid (stained with HEIDENHAIN's iron-alum haematoxylin). In this case it must be determined whether no centrosome exists or whether it existed but has been destroyed by some agency or other. So the following three questions must be settled, namely: a) Is the centrosome not destroyed by the fixative? b) Does the centrosome fail to appear owing to the physical state of the cell? c) Is the centrosome not observed owing to imperfect microscopical technique? (YUASA 1938). Concerning the question b), it is very difficult to obtain data about the physiological state of the cell, but by observing many materials which have been fixed and stained under various conditions it is possible to shed light on the problem whether or not the appearance or disappearance of the centrosome is affected by physiological conditions. So the present writer took the thalli of *Marchantia polymorpha,* which shows centrosomes in the cell at some stages, and fixed them after varying certain physiological condition such as the temperature, humidity or solar radiation. He also fixed them at various times of the day and in various seasons of a year. The thalli thus fixed are sectioned according to the paraffin method and stained with HEIDENHAIN's iron-alum haematoxylin. In the spermatogenous mitoses of these materials the writer could always observe the centrosome. Therefore the opinion that the presence or absence of the centrosome varies according to the physiological condition is not correct. According to FUJII and YASUI's experiment (1933) the presence or absence of the centrosome in the cells of higher or lower plants is not affected by treatment with solutions of varying pH-values. This experiment also affords evidence that the appearance of the centrosome is not affected by physiological conditions. The suitability of the microscopical technique is not an important question because imperfection of technique can be avoided by carefulness. So the most important problem among the above-mentioned questions is the effect of the fixative. According to the present writer's experiment (1938) the blepharoplast of the planocyte of *Fuligo septica* (basal body) which is thought to be homologous with the centrosome is always stained with HEIDENHAIN's iron-alum haematoxylin though it shows some variations of colour according to the fixative used, but sometimes it is destroyed by some kind of fixatives. Because of this experiment it is supposed that the centrosom is sometimes destroyed or rendered unstainable by certain fixatives. So the fact that IKENO (1903), BOLLETER (1905), M. WILSON (1911) and SHARP (1920) observed the centrosome in *Marchantia polymorpha, Conocephalus conicus, Pellia epiphylla* and *Blasia pusilla* respectively and that MIYAKE (1905) and ESCOYEZ (1907) could not observe the centrosome in *Marchantia polymorpha,* MIYAKE (1905), ESCOYEZ (1907) and WOODBURN (1911) in *Conocephalus conicus,* CHAMBERLAIN (1906) in *Pellia epiphylla* and WOODBURN (1911) in *Blasia pusilla,* are perhaps due to the effect of the fixative. Therefore when fixatives which do not render the centrosome unstainable are used, the centrosome must surely be stained with HEIDENHAIN's iron-alum haematoxylin when it exists. However, the centrosome is not always observed in the spermatogenous division of Eufilicinean plants when these are fixed with chrom-acetic acid solution (strong), chrom-acetic acid solution + distilled water (1:1), FLEMMING's solution (Bonn or weak), ZENKER's solution, ALLEN's solution, BENDA's solution (chondriosome method), CHMPY's solution (fixed for 24 or 48 hours) or REGAUD's solution which are thought not to have any destructive effects on the centrosomes or not to render them unstainable (YUASA 1938).

2) The present writer (1937, 1938) has often observed that the blepharoplast comes out from the spermatid nucleus in *Lygodium japonicum*, *Dryopteris oligophlebia* var. *elegans* and *Pteris multifida*.

3) In the fertilization of Eufilicinean plants and *Isoetes japonica* the border-brim and the lateral bar (often together with the cilia-bearing band) or the portion which has become differentiated from the spherical blepharoplast enter into the egg nucleus and fuse with the latter. This fact suggests the nuclear origin of the blepharoplast.

4) A species of bacteria, *Rhodospirillum longum*, has a diffuse nucleus. A basal body which is thought to be a primitive type of the blepharoplast occupies a portion of the diffuse nucleus and reacts positively to FEULGEN's nucleal staining, the presence of thymonucleic acid being thus shown. According to REICH (1926) the flagellum of the planocyte of *Stigeoclonium* develops from a body which has been derived from the caryosome. PICKARSKI (1937) stated that the blepharoplast of some Flagellatae shows a positive reaction to FEULGEN's nucleal staining. It is supposed that the blepharoplast of primitive type contains thymonucleic acid, but that in the history of its evolution the blepharoplast has acquired the power of derivation from the intranuclear substance, which is free from thymonucleic acid.

5) In *Stemonitis flavogenita* and *Fuligo septica* the blepharoplast (basal body) of the planocyte is derived from the nucleus and yet seems to have some relation to the nucleolus (YUASA 1935).

In Eufilicinean plants the centrosome is not observed in the spermatogenous division and the blepharoplast appears only in the spermatid (with the exception of *Nephrodium molle*): hence the blepharoplast is thought to be derived from the spermatid nucleus for the above-mentioned reasons. This conclusion may be applied, in general, to the blepharoplast of various plants. But to recognize its truth in the case of every plant which produces the blepharoplast every species must be studied. The present writer, however, wishes to maintain because of his own observations, that the blepharoplast in various plants is of nuclear origin.

Though the blepharoplast is thought to be derived from the nucleus, as it shows a negative reaction to FEULGEN's nucleal staining (YUASA 1935), it is clear that it contains no thymonucleic acid. Therefore the blepharoplast cannot have a direct relation with chromatin, but has a relation with nucleoplasmic matter other than chromatin. Then, from what nucleoplasmic matter is the blepharoplast derived?

2. THE BLEPHAROPLAST AND THE CHROMOSOME MATRIX

The nucleoplasm of resting nucleus, apart from chromatin is composed of nucleolus and nuclear lymph (or karyolymph). The chromatin

exists as chromonemata, while the chromosome matrix is thought to fill the nuclear cavity with another solution, forming the so-called nuclear lymph (SINOTÔ, unpublished). KUWADA (1937), referring to the nuclear lymph, remarked as follows: in the interphase the chromonemata are imbedded in the medium of the changed matrix and this medium, together with another fluid which has a different origin, is called nuclear lymph.

When the chromosomes are built up, being enveloped by the changed matrix the portion of the nuclear lymph other than the changed matrix does not stain with EEIDENHAIN's iron-alum haematoxylin, no matter what fixative is used for its fixation. Whereas the blepharoplast is always stained with HEIDENHAIN's iron-alum haematoxylin, no matter what fixative is used, with the exception of the cases when it is destroyed by the fixative. The nuclear lymph other than the changed matrix never changes irreversibly into the gel state, while the blepharoplast, at least the border-brim, tends to take on the gel state in the early stages of its development and reverts again to the sol condition in the egg nucleus in fertilization. So the blepharoplast is thought to change irreversibly into the gel state. These facts indicate no similarity between the blepharoplast and nuclear lymph and it is denied that the blepharoplast originates from the portion of the nuclear lymph other than the changed matrix. Then, is there any relation between the matrix and blepharoplast?

In the stage between the telophase and the spiral stage of the next division the old chromonemata do not disappear and again show spirals in the newly formed cells, while the matrix also exists, showing reversible changes (KUWADA 1937). When the chromonemata are changed into the chromosomes the former are enveloped by the matrix and it is unnatural to suppose that a portion of the matrix comes from the nucleus and becomes the blepharoplast. Moreover there are the following differences between the nature of the matrix and blepharoplast:

Matrix	Blepharoplast
1. Sometimes it is stained with HEIDENHAIN's iron-alum haematoxylin, but very faintly.	1. It is stained deeply with HEIDENHAIN's iron-alum haematoxylin.
2. It is stained faintly with an aqueous solution of gentian violet.	2. It is stained deeply with an aqueous solution of gentian violet.
3. It is destroyed by boiling water.	3. It is destroyed with difficulty by boiling water.

When the planocytes of *Fuligo septica* are stained with nucleus staining or cytoplasm staining dyes the differences of colouration are seen between the blepharoplast (basal body) and the nuclear lymph (Table II). In the living nucleus the nuclear lymph is stained with acid dyes, such as aniline blue, erythrosin, eosin or light green, while the chromatin sub-

stance is not. Basic dyes do not stain the nuclear lymph but stain the chromatin substance. The blepharoplast hardly stains with acid dyes, while it stains easily with basic dyes.

As stated above, the existence of a relationship between the blepharoplast and nuclear lymph is denied, but is there any relation between the blepharoplast and spindle substance which is thought by some authors to be included in the nucleus (SCHUSSNIG 1930, KUSANO 1930, SELIM 1931, WADA 1935)? There are some differences of colouration between the blepharoplast and spindle substance, because the spindle is stained clearly with HEIDENHAIN'S iron-alum haematoxylin only when it is fixed with a fixative which contains a large quantity of acetic acid, while the blepharoplast is always stained even when fixed with a fixative which contains no acetic acid. According to WADA (1935) the spindle substance is an intranuclear product and is thought not to fuse with the cytoplasm, for when a hole is made in the cell membrane some or all the spindle substance comes out from the cell through this hole independently from the cytoplasm, together with the chromosomes.

Therefore the existence of a relationship between the spindle substance and blepharoplast must be denied.

3. THE BLEPHAROPLAST AND NUCLEOLUS

The relation between the blepharoplast and nucleolus has been discussed by various authors. IKENO (1930) observed that the centrosome, at first, comes out from the nucleus as the spermatogenous division is beginning, divides into two bodies and takes part in the spermatogenous division and that the centrosome becomes the blepharoplast in the spermatid. According to DAVIS (1908) the nuclear substance comes from the nucleus and forms a ring-shaped blepharoplast in *Derbesia*. The nuclear origin of the blepharoplast was also observed in *Mnium, Fegatella, Conocephalus* and *Polytrichum* by VAN LEEUWEN-REIJNVAAN (1908) and in *Dioon* by CHAMBERLAIN (1909). Most of these authors suggested a relationship between the blepharoplast and nucleolus. According to M. WILSON (1911) the blepharoplast originates from the nucleolus of the spermatid nucleus in Bryophyta. BAGCHEE (1924) showed in *Anthoceros laevis* that the blepharoplast was derived from a fragment of main portion of the spermatid nucleus. From because of the similarity in microchemical characteristics of the border-brim and nucleolus LANG (1936) supported the view that the blepharoplast originates from the nucleolus.

The present writer (1934, 1937) had already observed in *Stemonitis* and *Fuligo* that the blepharoplast and centrosome belong to one and the same double structure and that in the spore germination of *Fuligo septica*

the blepharoplast originates from the nucleus, in relation with the nucleolus (1935, 1936). In the planocytes of *Stigeochronium* REICH (1926) observed the fact that the flagellum originates from a portion of the caryosome. Many similarities are seen between the blepharoplast and nucleolus as shown below:

1. The nucleolus and blepharoplast stain almost in the same shade with HEIDENHAIN's iron-alum haematoxylin, no matter what fixative is used. With aceto-carmine both bodies swell somewhat and stain faintly. Both bodies were stained in the same shade by BIONDI-EHRLICH's, AUERBACH's, SCHAEDE's or UNNA-PAPPENHEIM's method which can stain the nucleolus differently from the chromosome. Both bodies stain vitally in the same shade with Prussian blue or neutral violet. Both bodies are negative to FEULGEN's nucleal staining reaction [with the exception of the cases in which the nucleolus is positive to the reaction, namely, in Cucurbitaceae, *Ricinus communis* (YAMAHA and SUEMATSU 1936), *Trichomanes orientalis, Ceratopteris thalictroides, Ceratopteris* sp., *Lygodium japonicum, Ophioglossum ellipticum* and *Lycopodium* sp. (YUASA 1936). The blepharoplast of *Rhodospirillum longum* (YUASA 1936) and some Flagellatae show a positive nucleal reaction (PICKARSKI 1937)].

TABLE I

Nucleus staining dyes	Chromatin substance	Nuclear membrane	Nucleolus	Nuclear lymph	Blepharoplast	Remarks
1% aqueous solution of gentian violet	++	+	++	+	++	Fixed with CARNOY' fluid (3:1).
Safranin dissolved in aniline water	++	−	++	+	++	In vivo.
HEIDENHAIN's iron-alum haematoxylin	+++	+	+++	−	+++	Fixed with chrom-acetic acid solution. Paraffin method.
1% aqueous solution of safranin	++++	++	++++	++	++++	In vivo or fixed with CARNOY's fluid.
1% alcoholic (95%) solution of safranin	+++	++	++++	++	++++	In vivo.
Acid alizarin blue (chrom-alum)	±	±	±	±	±	In vivo.
Aceto-carmine	++	−	+	+	+	In vivo or fixed with CARNOY's fluid.
Acetic acid solution (45%) of methylen-blue	+++	−	−	−	−	Fixed with CARNOY's fluid.
EHRLICH's triacid	+	+	++	+	++	In vivo.
Alcoholic solution (70%) of sudan III	+	+	+	+	+	Fixed with CARNOY's fluid.

In *Cyrtomium falcatum* the nucleolus of the prothallium cell and the blepharoplast in the spermatid stain in the same shade with the nucleus staining dyes as shown in Table I.

2) Physico-chemical properties. According to SHINKE and SHIGENAGA (1933) the nucleus contains lipoids. The present writer proved that in *Cyrtomium falcatum* the nucleoli of the prothallium cell contain lipoids and so also does the blepharoplast. MILLON's reaction was positive in the case of the blepharoplast and nucleolus.

In *Cyrtomium falcatum* the nucleolus gradually swells and dissolves in n HCl (the nuclear membrane and chromatin substance show almost no changes). The same phenomenon can be recognized in the case of the blepharoplast. The chromatin substance is gradually destroyed by hot water at 60–90°C, while the nuclear membrane and nucleolus remain unchanged. The blepharoplast, especially the one which has begun to elongate, is strongly resistant to hot water.

The nucleoli of the higher plants are stable and resistant to alkali salts, oil-dissolving medium, fuming HCl, or chromic acid (50%) (YAMAHA 1927). The same facts can be recognized in the case of the blepharoplast of the spermatozoid of Polypodiaceae.

The nucleolus dissolves in pepsin-HCl solution, dilute HCl (YAMAHA 1927) and disappears when treated with chloroform (MASACRÉ and PICARD 1933): the blepharoplast also shows almost the same reactions.

GEITLER (1936) remarked that in the mitosis of *Cladophora* the old nucleus and the newly formed one are stained differently. A similar fact can be observed in the case of the blepharoplast which has differentiated into the border-brim and cilia-bearing band: the border-brim stains deeply with HEIDENHAIN's iron-alum haematoxylin, while the cilia-bearing band stains faintly.

3) In the planocytes of *Fuligo septica* which have been immersed in hot water at 60°C, dried up on a slide and stained with HEIDENHAIN's iron-alum haematoxylin the cytoplasm does not stain, the nucleus stains faintly and the nucleolus and the blepharoplast stain somewhat deeply. When the planocytes are dried gradually with n HCl on a slide both the nucleolus and blepharoplast dissolve. The stainability of the nucleolus and blepharoplast is as follows (Table II).

4) In Pteridophyta and Bryophyta the cell has nucleoli in its nucleus generally, but there are no nucleoli in the nucleus of the spermatid. The absence of nucleoli in the spermatid is thought to suggest the extrusion of nuclear substance from the nucleus in order to form the blepharoplast. After fertilization, however, the egg nucleus contains two nucleoli, in general (SHOWALTER 1928, YAMANOUCHI 1908, YUASA 1937), one of which may be thought to have been derived from the blepharoplast of the spermatozoid.

TABLE II

Nucleus staining dyes	Nucleus			Blepharoplast (basal body)	Bell-jar shaped portion between blepharoplast and nucleus	Flagellum	Cytoplasm	Chondriosome	Rhizoplast	Remarks
	Chromatin granules	Nuclear lymph	Nucleolus							
1% aqueous solution of gentian violet	+++	+	+++	+++	—	+	+	+++	±	In vivo.
Safranin dissolved in aniline water	+	+	++	++	—	—	—	+		In vivo.
HEIDENHAIN's iron-alum haematoxylin	+++	—	+++	+++	—	—	—	+++	++	Dried preparation.
1% aqueous solution of safranin	+	—	+	+	—	—	—	+		In vivo.
1% alcoholic (95%) solution of safranin	++	+	++	++	—	—	—	++		In vivo or fixed with osmic acid.
Alizarin-cyanin	—	—	Dark violet, very faintly	Dark violet, very faintly	—	—	—	Dark red, very faintly	—	Fixed with osmic acid.
Aceto-carmine	Red	Red	Dark red	Dark red	—	—	Faint red	—	—	In vivo.
EHRLICH's triacid	Faint blue	Faint blue	Dark violet	Dark violet	—	+	—	Dark violet	Dark violet	Fixed with osmic acid.

Cytoplasm staining dyes	Nucleus			Blepharoplast (basal body)	Bell-jar shaped portion between blepharoplast and nucleus	Flagellum	Cytoplasm	Chondriosome	Rhizoplast	Remarks
	Chromatin granules	Nuclear lymph	Nucleolus							
1% aqueous solution of acid fuchsin			Dark red	Dark red						Homogeneously stained. (Dark red)
1% aqueous solution of eosin	Red	Red	Dark red	Dark red	Faint red	Faint red	Faint red	—	—	In vivo.
1% aqueous solution of aniline blue	—	—	+	+	—	—	—	—	—	Dried preparation.

5) The border-brim is a portion which has been differentiated from the blepharoplast, and on the basis of morphological study it is thought to be a portion which retains many physico-chemical properties of the blepharoplast in the early stages. As already shown by the present writer (1936) the border-brim of the spermatozoid in Polypodiaceae contains lipoid and protein. This fact coincides with the fact that *the nucleolus of Cyrtomium falcatum contains lipoid and protein.* In the spermatozoid of *Isoetes japonica* the border-brim is not destroyed by drying and is not changed morphologically with 40% NaOH or 33% HNO_3, but dissolves when it is heated with 40% NaOH or 33% HNO_3. These properties of the border-brim resemble those of the nucleolus. The nucleoli of the prothallium cell stain with nucleus staining or cytoplasm staining dyes in the same shade as the border-brim (YUASA 1936). From the experiments involving the treatment with boiling water, 8% HCl or aqueous solution of sodium borate LANG (1936) concluded that the border-brim and blepharoplast resemble in their properties the substance which forms the nucleolus and that the border-brim is composed of nucleolin.

6) The nucleolus contains two kinds of substances (ZIRKLE 1931). According to the state of the existence of these two substances the nucleolus takes on various shapes suggestive of that of the blepharoplast. The nucleolus disintegrates into fragments (BARANOV 1926, SENJANINOVA 1926, SOROKAN 1927, 1929) and fuse to each other (SCHAEDE 1929, PEKAREK 1932) (cf. LANG 1936). The nucleolus often comes out from the nucleus, disappearing in the cytoplasm (PRISCILLA and BROWN 1930) or forming the extra-nuclear nucleolus (YAMAHA and SINOTÔ 1925). This fact shows that the nucleolus can be divided.

All these facts show the resemblance between the nucleolus and the blepharoplast. But it may be overhasty to conclude that the blepharoplast originates from the nucleolus. Though the nucleolus can be extruded out of the nucleus under abnormal conditions, for example, as a result of the action of centrifugal force (BEAMS and KING 1931), some authors (ZIRKLE 1931, HEITZ 1931) have denied that the nucleolus comes out from the nucleus in normal conditions or that the plastin which is contained in the nucleolus goes to the pole of the spindle. However, because the blepharoplast originates from the nucleus and resembles nuclear substance in its nature it is supposed that the blepharoplast may originate from the nucleolar substance, even if not from the nucleolus itself.

4. BLEPHAROPLAST AND CYTOSOME[1]

MOTTE (1928) stated that the blepharoplast may be of chondriosome nature and DRACINSCHI (1932) also showed the chondriosome nature of

(1) Cytosome means the portions of the cell other than the nucleus and the contents of the cytoplasm.

the "Randsaum" (the border-brim of the writer). LANG (1936), however, objected to this opinion because of the fact that the blepharoplast is preserved when stained with aceto-carmine.

The present writer (1937, 1938) recognized the fact that in Polypodiaceae and *Isoetes japonica* the border-brim resembles the chondriosome in its stainability, but in Polypodiaceae he showed the constant number and size of the chondriosomes during the course of spermatoteleosis and yet could trace the behaviour of chondriosomes independently from that of the blepharoplast. EMBERGER (1922) also observed the mutual independence of the chondriosomes and the blepharoplast during the course of spermatoteleosis. From these facts it may be concluded that the blepharoplast is different from the chondriosome.

Then it may be supposed that the blepharoplast is perhaps homologous with the plastid. The plastid, however, can be traced independently from the blepharoplast during the course of spermatoteleosis and is thought to be different from the blepharoplast (YUASA 1937, 1938).

The belief that the blepharoplast is produced from the dense portion of the cytoplasm ("plasmodermale Blepharoplasten") (STRASBURGER 1892, BELAJEFF 1894, MOTTIER 1904) coincides with the one that the blepharoplast appears *de novo* in the cytoplasm (cytoplasmic or endoplasmic blepharoplast) (GUIGNARD 1898, WEBBER 1901, HIRASE 1898, SHAW 1898, BELAJEFF 1898, ESCOYEZ 1907, WOODBURN 1911, HUMPHREY 1905, WOODBURN 1911, 1920) when viewed from the stand-point of the cytoplasmic origin of the blepharoplast. However, the absence of a homologous relationship between the cytoplasm and blepharoplast is presumed from the staining reaction and physico-chemical properties of the two. The possibility that the blepharoplast comes out from the cytoplasm and changes its cytoplasmic properties must also be dismissed, because the stage during which the blepharoplast is coming out from the nucleus has been observed by the present writer (YUASA 1937, 1938). The cytoplasmic blepharoplast is one which comes out from the nucleus and is situated in the cytoplasm far apart from the nucleus.

The correctness of the theory that the blepharoplast originates from the centrosome (centrosomal blepharoplast) (BELAJEFF 1898, 1899, SHARP 1912, 1914, 1920, IKENO 1903, SCHAFFNER 1908, BOLLETER 1905, WILSON 1911, LEWIS 1909, JAHN 1904) is recognized by most of the recent authors. According to this theory it is assumed that the centrosome and blepharoplast belong to one and the same double structure which acts as a centrosome in spermatogenous divisions, while as a blepharoplast in the spermatid (HENNEGUY 1898, WILSON 1928). The double structure is observed in many cases (PROWAZEK 1904, ALEXEIEFF 1924, KUCZYNSKI 1918, BĚLAŘ 1921, DOBELL 1908, DOFLEIN 1918, KOFORD and SWEZY 1915, LACKEY 1933). It can also be proved from

the similarity of the physico-chemical properties of the blepharoplast and the centrosome.

In these cases the blepharoplast is derived from the centrosome, therefore the origin of centrosome must be considered. This problem will be discussed in Chapter IV.

5. The origin of the blepharoplast and its universality

It is assumed from the following facts that in the plants where the double structure appears only as the blepharoplast it has a nuclear origin and that it is derived from the intranuclear substance which is similar in nature to the nucleolus forming substance. The facts are:

1) The similarity of blepharoplast and nucleolus with regard to stainability.
2) The similarity of blepharoplast and nucleolus with regard to physico-chemical properties.
3) The blepharoplast of the planocyte in Myxomycetes is derived from the nucleus in relation with the nucleolus.
4) No nucleoli can be observed in the spermatid nucleus, but two are present in the nucleus of the fertilized egg.
5) The similar characteristics of the border-brim and nucleolus.
6) The power possessed by the nucleolus of changing its form, dividing and fusing.
7) No relationship can be presumed to exist between the blepharoplast and the nucleoplasm other than the nucleolus.

According to Zirkle (1931) plastin is contained both in the chromosome and nucleolus and can be stained differently from the chromatin substances by using certain special fixatives. Lang (1936) showed that the blepharoplast is composed of plastin-like substances. But it would be hasty to presume from these observation that the blepharoplast comes out of the nucleolus itself.

The present writer only concludes that the substance which is homologous in nature with the nucleolus forming substance comes out from the nucleus, becoming the blepharoplast and that the blepharoplast has its origin in the nucleolar substance though the mechanism of production and extrusion of this substance from the nucleus could not be made clear in the present study. This idea is designated the nucleolar substance theory.

The fact that the centrosome is homologous with the blepharoplast and that the blepharoplast has its origin in the nucleolar substance can be demonstrated by observation on various plants as already shown. And as the structure of the blepharoplast is thought not to be basically

different in various plants, the nucleolar substance theory may be applied to almost all the plants which show a blepharoplast during the life-cycle.

The relationship between the nucleolus and the special portion of the chromosome matrix has been considered by some authors and HEITZ (1931, 1932) stated that the nucleolus appears, at first, in the constricted portion of a certain definite chromosome, while MATSUURA (1935) stated that a certain portion of the chromosome other than the chromonemata has some relation with the nucleolus. Therefore the nucleolar substance exists not only in the nucleolus itself but also in some portion of the nucleus other than the nucleolus.

IV. The origin of the centrosome

As shown above the blepharoplast is supposed to originate from the nucleus, having some relation with the intranuclear substance which is similar in nature with the nucleolus. Then what is the origin of the centrosome? If it is recognized that the centrosome and blepharoplast belong to one and the same double structure, the centrosome must also be thought of as derived from the intranuclear substance. This problem will be discussed in the following. First of all, the ways in which the double structure of the blepharoplast and centrosome may appear can be reduced to three in number for the purpose of classification, viz:—

1) The double structure may act as a centrosome, having no function as a blepharoplast. In this case only the centrosome appears and no blepharoplast is seen.

2) The double structure may act as a blepharoplast, having no function as a centrosome. In this case only the blepharoplast appears and no centrosome is seen.

3) The double structure may act both as a centrosome and a blepharoplast. In this case both the blepharoplast and centrosome appear.

1. THE DOUBLE STRUCTURE ACTING AS CENTROSOME

The centrosomes were observed in the mitoses in the oogonia of *Achlya polyandra* (MÜCKE 1908), *Saprolegnia* (TROW 1904), and *Fucus vesiculosa* (YAMANOUCHI 1909), in the developmental mitoses of *Dictyota dichotoma* (WILLIAMS 1904), in the spermatogenesis of *Helvella crispa* (CARRUTHERS 1911), in the sporogenesis of *Humaria granulata*, *Ascobolus furfuraceus* and *Lachnea stercorea* (FRASER and BROOKS 1909), *Gelasinospora tetraspora* (DODGE 1937), *Peziza vesiculosa* (WELSFORD 1908) and Uredineae (BLACKMANN 1904), in the mitoses of the vegetative cells of *Dictyota* sp. (WILLIAMS 1904), in the mitoses of the tetraspore

mother cell of *Corallina officinalis* (DAVIS 1898), in the mitoses of the embryo of *Preissia quadrata* (GRAHAM 1918) and the mitoses of *Nymphaea alba, Nuphar luteum, Lymodorum abortivum* (GUIGNARD 1898), *Allium Cepa* and *Sagittaria variabilis* (SCHAFFNER 1898). The cases observed by GUIGNARD (1898) and SCHAFFNER (1898), however, are not generally recognized by recent investigators.

In all these cases the centrosomes appear at the beginning and disappear at the end of the mitoses. In sporogenesis, however, the centrosome remains in the spore cells even at the end of the division and fulfills a function as the initial point of the spore membrane.

From the results of the studies mentioned above the behaviour of the centrosome is supposed to be as follows. In the beginning of the mitosis the centrosome appears at the contact region of the cytoplasm and nuclear membrane at one pole of the nucleus and produces many astral rays. Before the appearance of the centrosome a special kind of cytoplasm seems to be organized at one polar region of the cell in which the centrosome appears (DAVIS 1898). The centrosome divides into two bodies, one of which moves towards the opposite polar region of the cytoplasm. So one centrosome is seen at each polar regions of the cytoplasm. At this stage the nucleus becomes slender between the two poles and, at extreme cases, spindle-shaped (WILLIAMS 1904, DAVIS 1898). From this transformation of the nucleus it is supposed that physicochemical change has occurred both within and without the nucleus and especially at the polar region of the latter.

There is no reason to deny the truth of the conception that the astral rays around the centrosome provide passage for the protoplasmic substance, because it is natural to presume that passages for transfer of the protoplasmic substance appear owing to the colloid chemical changes in the polar region of the cell. But it is also supposed that the polar region containing the centrosome is pulled by all the astral rays and becomes the fixed portion. These two fixed portions pull the nucleus from opposite positions and provide stimuli for formation of the spindle by the intranuclear spindle substance.

The astral rays around each centrosome, which face the nucleus, penetrate, at last, into the nucleus from both the polar regions of the nucleus. At this stage the polar regions of the nuclear membrane dissolve away and the cytoplasm of the fixed portions seems to come into contact directly with the intranuclear spindle substance at each pole of the cell.

When colloid chemical changes occur at one polar region of the nucleus before mitosis a centrosome is presumed to be derived from the nucleus. This conclusion is confirmed by vital observation as stated later. The fixed region of the cytoplasm near the pole of the cell is pulled from all sides with the exception of the side where it faces toward the nucleus.

However, as the fixed region adheres closely to the nucleus, the latter is indirectly pulled by the former at the polar region. As a result some substance is extruded from the nucleus into the fixed region of the cytoplasm through the nuclear membrane. A similar phenomenon can be observed in the course of spermatoteleosis in Pteridophyta where the blepharoplast comes out from the spermatid nucleus, elongates, comes into contact with the nucleus and sucks some substance from the nucleus through the nuclear membrane, completing the border-brim, lateral bar and cilia-bearing band. The emergence of the centrosome from the nucleus was observed in *Marchantia polymorpha* by IKENO (1903) and in *Mnium hornum* and *Atrichum undulatum* by WILSON (1911).

In some cases the centrosome is already organized in the nucleus and comes out from the latter to take part in mitosis. For example, in the mitosis of ascogenous mycelium of *Peziza vesiculosa* (WELSFORD 1908) two centrosomes appear at one pole in the nucleus and one of these moves towards the antipole to take part in the mitosis. That is to say, the centrosome appears, in this case, in the nucleus. The spindle is also thought to be intranuclear (DODGE 1937, WADA 1935). In some cases both the centrosome and spindle develop in the nucleus (*Surirella calcarata* by LAUTERBORN after SCHUSSNIG 1938) and in other cases the centrosome is always situated interior to the nuclear membrane (*Achlya polyandra* by TROW 1904, *Peziza vesiculosa* by WELSFORD 1908, *Vaucheria sessilis* by HANATSCHEK after SCHUSSNIG 1938).

In *Corallina officinalis* (DAVIS 1898) a portion of the kinoplasmic substance which takes part in the formation of the centrosphere is again taken into the newly formed daughter nucleus at the telophase of the mitosis. In the sporogenesis of some fungi the centrosome of the previous division remains connected with the nucleus and acts as the initial point of the spore membrane. These facts also suggest a relationship beween the centrosome and nucleus [*Helvella crispa* by CARRUTHERS (1911), *Humaria granulata, Ascobolus furfuraceus* and *Lachnea stercorea* by FRASER and BROOKS (1909), Uredineae by BLACKMAN (1904), *Gelasimospora tetraspora* by DODGE (1937) and *Pustularia balarioides* by BAGCHEE (1925)]. In *Helvella crispa* CARRUTHERS (1911) observed that in the early stage of reduction division the nuclear substance is often extruded from the nucleus. This fact is also related with the nuclear origin of the centrosome. When once the centrosome is organized in the cytoplasm it is not difficult to suppose that the cytoplasm around the centrosome often shows a very complicated structure.

The present writer observed the behaviour of the centrosome *in vivo* and in fixed and stained preparations in *Dictyota dichotoma, Neurospora crassa, Peziza* sp. and *Pyronema* sp. and presumed the origin and behaviour of the centrosome to be as follows, taking into consideration

the above-mentioned facts, namely at the beginning of mitosis special cytoplasm appears at one polar region of the cell, showing the effect of the colloid chemical changes at this stage. Soon, this region radiates cytoplasmic fibers on all sides with the exception of the side which faces the nucleus. These radial fibers are, however, very difficult to observe in the vital state, but when fixed they become easily visible, being designated as the astral rays. The special cytoplasm is thought to be pulled by these astral rays and to become the fixed region. In some cases the nuclear surface which makes contact with the fixed region of the cytoplasm swells out as a small protuberance. In this state the nucleus resembles very much in appearance the developing planocyte of *Fuligo septica*, *Stemonitis flavogenita* or *St. splendens* var. *flaccida*, where the blepharoplast comes out from the nucleus.

The above-mentioned protuberance may be a portion of the nucleus through which the nuclear substance comes out to form a centrosome. Soon the protuberance is withdrawn again. After this stage the fixed region divides into two portions. Therefore it is supposed that some nuclear substance comes out from the nucleus through the protuberance and the cytoplasm around that nuclear substance is organized to divide into two portions. One portion of the fixed cytoplasm moves towards the opposite pole, containing a centrosome. The process by which this movement takes place could not be clearly followed.

Here a fixed region, having a centrosome in its center, is seen at each pole of the cell and the nucleus is stretched between these fixed regions. It is by means of this stretching force that the spindle substance is thought to be arranged in the nucleus. Then the spindle is formed and the polar portions of the nucleus comes in contact directly with the cytoplasm as the nuclear membrane disappears, at first, at the polar portions of the nucleus. In some cases, however, the nuclear membrane remains unchanged after the spindle is formed.

In the plants where the centrosomes appear during the mitosis of their cells it is presumed that one polar region of the nuclear membrane comes to take on a special character in the beginning of mitosis that allows passage of the nuclear substance out of the nucleus to form a centrosome. In the plants which have no centrosomes in the mitosis of their cells, however, this change in the nature of the nuclear membrane may not take place, and hence the nuclear substance cannot come out to form a centrosome. In the latter case the fixed regions appear, at first, not only at one polar region of the cell but also at the antipodal region of the cell and pull the nucleus from each pole. The nuclear membrane, however, seems to undergo no change in its nature. As stated by WADA (1935) in the stamen hair cells of *Tradescantia*, when the mitosis begins the

nucleus becomes spindle-shaped as a whole to form the spindle in which the chromosomes are involved.

In the cells where centrosomes appear during mitosis the centrosome is thought to be derived from the nucleus, so according to the ways by which it comes out from the nucleus it takes a granular form (*Stypocaulon scoporion* by SWINGLE after SCHUSSNIG 1938), a spherical form (*Achlya* by TROW 1904; *Fucus* by YAMANOUCHI 1909), a large spherical form (*Corallina* by DAVIS 1898; *Cycas revoluta* by IKENO 1898) or a rodlet form (*Peziza subumbrina* by MATSUURA and GONDO 1935).

In the higher plants the appearance of the centrosome (*Nymphaea alba, Nuphar luteum* and *Limodorum abortivum* by GUIGNARD 1898, *Allium Cepa* and *Sagittaria variabilis* by SCHAFFNER 1898) has not been generally recognized, so the discussion of such cases has been omitted here.

2. THE DOUBLE STRUCTURE ACTING AS BLEPHAROPLAST

In this case the classification must be made into the following two categories:

a) The blepharoplast appears in the cell which is directly transformed into the flagellated cell.

This case was already discussed in Chapter III and it is assumed that the blepharoplast originates from the nucleus.

b) The blepharoplast appears in the cells which divide to make spermatids.

In these cases the blepharoplast appears in the spermatogenous cell or spermatid mother cell, but does not act as a centrosome. These cases were observed in *Marsilia quadrifolia* (SHAW 1898), *Dioon edule* (CHAMBERLAIN 1909), *Zamia integrifolia* (WEBBER 1897) and *Microcycas calocoma* (CADWELL 1907). In most of these cases, however, the bodies which were thought to be the blepharoplasts of spermatogenous cells or spermatid mother cells were proved by other authors to be the centrosomes, so it must be considered that in these cases the double structure acts as both centrosome and blepharoplast. Accordingly these cases must be discussed in this Chapter 3. In *Nephrodium molle* (YAMANOUCHI 1908), however, the blepharoplast appears at first in a spermatid mother cell and divides into two, each one of which enters into the resulted spermatid. So this case may be included in this Chapter 2 a.

3. THE DOUBLE STRUCTURE ACTING AS CENTROSOME AND BLEPHAROPLAST

These cases are observed in Myxomycetes, Bryophyta, Pteridophyta and Gymnospermae, in which the double structure acts both as the centro-

some and blepharoplast as observed in *Marchantia polymorpha* (IKENO 1903), *Mnium, Fegatella, Conocephalus* and *Polytrichum* (VAN LEEWEN-REIJNVAAN 1908), *Mnium hornum* and *Atrichum undulatum* (WILSON 1911).

A centrosome appears at the beginning of the spermatogenous mitosis and disappears at the end of the latter. It is generally recognized that in the last division the centrosome remains in the spermatid to become transformed into the blepharoplast (*Marsilia quadrifolia* by SHARP 1914; *Equisetum arvense* by SHARP 1912; *Marchantia polymorpha* by IKENO 1903).

The process by which the centrosome appears in the spermatogenous cell is thought to be the same as that shown in this Chapter 1. In the next division a centrosome again appears from the nucleus, but it is not clear whether the centrosome of the previous division again comes out from the nucleus or whether a new one appears. Judging from the vital observation or that of fixed and stained preparations of *Marchantia polymorpha* made by the present writer the centrosome may be presumed to enter into the newly formed daughter nucleus at the end of the mitosis.

In *Marchantia polymorpha* the centrosome is often situated in the cytoplasm apart from the nucleus and divides into two. This condition is very suggestive of a cytoplasmic origin for the blepharoplast. In this case, however, the centrosome must come out from the nucleus and move apart from the latter to divide into two bodies. The extrusion of the nuclear substance from the nucleus to form the centrosome must be due to the action of the fixed region of the cytoplasm as discussed in this Chapter 1.

As stated above, the disappearance of the centrosome into the newly formed daughter nucleus is sometimes noticed in the case of vital observation or that of fixed and stained preparations of *Marchantia polymorph.* A similar phenomenon was observed in the planocytes of *Stemonitis* in which the blepharoplast of one planocyte enters into the nucleus of the other one during the process of conjugation and the blepharoplast is sucked up into the nucleus when the planocyte is transformed into the cyst state.

Now the double structure acts as the blepharoplast in the spermatid, losing its function as the centrosome. The process by which the organization of centrosome is transformed into that of blepharoplast is not made clear in this study. The fact that during spermatoteleosis the blepharoplast often divides into several granules (SHARP 1912, 1914, 1920; IKENO 1898; YUASA 1934, 1939; DAVIS 1908) suggests that the blepharoplast still retains the power of division (cf. SHARP 1920).

Some authors maintain that the cilia arise from the astral rays around the centrosome, but the present author observed that in *Fuligo*

and *Stemonitis* the flagellum develops from the blepharoplast (YUASA 1935, 1937) and that in Eufilicinean plants the cilia appear as processes upon the surface of the cilia-bearing band (YUASA 1933).

In Eufilicineae the blepharoplast occupies the anterior portion of the spermatozoid and is taken into the egg nucleus during fertilization: the cilia-bearing band is, however, often destroyed during the fertilization.

V. The centrosome and the nucleolar substance theory

It has already been assumed, as shown above, that when the blepharoplast appears in the mother cell of the flagellated cell it comes out from the nucleus and, moreover, from a substance similar in nature to the nucleolar substance. As the centrosome and blepharoplast are thought to belong to one and the same double structure it is natural to suppose that the centrosome may also be derived from the nuclear substance. But direct evidence that the nucleolar substance produces the centrosome was not found in this study and the final conclusion must be deferred until a future investigation will be done.

VI. Mitosis without a centrosome

It is generally found that in most of the mitoses and reduction divisions of plants the centrosome does not appear. According to the vital observation of the cells of the root-tips of *Allium Cepa* and *Vicia Faba* and of the pollen mother cells of *Plantago lanceolata* the special cytoplasm becomes differentiated at both the polar regions of the cell in the later prophase. This special cytoplasm appears at each polar region of the cell, but has no astral rays around it. Accordingly this special cytoplasm is not pulled firmly from all sides and is a relatively weak fixed region. Therefore this fixed region is thought not to have sufficient force to cause extrusion of the nuclear substance into the cytoplasm.

This fixed region is designated in some cases as the kinoplasmic cap or polar cap. The nucleus is pulled between the two fixed regions, but the nuclear membrane is supposed not to be changed in nature to extrude nuclear substance. As the nuclear substance does not come out from the nucleus the centrosome does not appear in the mitosis. The fixed region of the cytoplasm has, however, been supposed by some authors (cf. BĚLAŘ 1927; GEITLER 1934) to correspond to the centrosome.

The nucleus becomes elongated as a whole into a spindle-shaped body to complete the mitotic figure. The nuclear membrane of the polar portions of the spindle-shaped nucleus dissolves at first, and comes into direct contact with the fixed region. The spindle substance in the nucleus

completes the spindles and the nuclear membrane gradually disappears. When in a wide area the nuclear membrane of the polar regions of the spindle-shaped nucleus dissolves the poles of the spindle thus formed are very broad as was shown in the case of *Spirogyra* (GEITLER 1934) or in the pollen mother cell of *Carex grallatoria* var. *heteroclita* (TANAKA 1939).

VII. Conclusions

1. In the plant kingdom the centrosome and the blepharoplast are thought to belong to one and the same double structure.

2. When the double structure appears as the blepharoplast in the cell which is transformed directly into the flagellated cell it is derived from the nucleus and moreover from an intranuclear substance similar in nature to the nucleolar substance. This concept is designated the nucleolar substance theory.

3. When the double structure appears as the centrosome it is also derived from the nucleus. Whether the centrosome is derived from the nucleolar substance or not was not determined in this study.

4. Even when the double structure appears both as the centrosome and blepharoplast it is derived from the nucleus in the beginning and disappears into the daughter nucleus at the end of the spermatogenous mitosis. The centrosome of the last spermatogenous mitosis remains in the spermatid and plays the rôle of blepharoplast.

5. The absence of a centrosome in the mitosis may be due to the fact that the nuclear substance does not come out from the nucleus in the beginning of the spindle formation owing to the weak force of the fixed region and the absence of change in the polar region of the nuclear membrane.

The writer wishes to express his sincere appreciation for the valuable aid of Director Dr. H. HATTORI of the Tokugawa Institute for Biological Research. Thanks are also due to Prof. Y. SINOTÔ and Dr. B. WADA of Tôkyô Imperial University for their valuable suggestions.

The expenses for carrying out this study were partly defrayed out of a grant from the "Nippon-Gakuzyutu-Sinkôkwai" (The Japan Society for the Promotion of Scientific Research), to which the writer wishes to express his best thanks.

THE TOKUGAWA INSTITUTE FOR
BIOLOGICAL RESEARCH

VIII. Bibliography

ABEL, K. 1930. Untersuchungen über die Nukleolen. Protoplasma **10**: 550–567.

ALEXEIEFF, A. 1924. Sur la question du noyau chez les bactéries. Contribution à l'étude des mitochondries et des grains métachromatiques. Arch. f. Protistenk. **49**: 396–432.

ALLEN, C.E. 1912. Cell structure, growth and division in the antheridia of *Polytrichum juniperinum* WILLD. Arch. f. Zellf. **8**: 121–188.

BAGCHEE, K. 1924. The spermatogenesis of *Anthoceros laevis* L. Ann. of Bot. **38**: 105–112.

——— 1925. Cytology of the Ascomycetes. *Pustularia bolarioides* RAMSB. I. Spore formation. Ib. **39**: 217–266.

BARANOV, P. 1926. Cytologische und embryologische Untersuchungen über *Drimiopsis maculata*. Zeit. f. Zellf. u. mikr. Anat. **3**: 131–148.

BEAMS, H.W. and KING, R.L. 1931. Effect of ultracentrifuging on the cells of the root tip of the bean. Nature **135**: 232.

BELAJEFF, W. 1888. Über Bau und Entwickelung der Spermatozoiden bei den Gefässkryptogamen. Ber. Deut. bot. Gesells. **7**: 122–125.

——— 1894. Über Bau und Entwicklung der Spermatozoiden der Pflanzen. Flora **79**: 1–48.

——— 1897. a. Über die Spermatogenese bei den Schachtelhalmen. Ber. Deut. bot. Gesells. **15**: 339–342.

——— 1897. b. Über den Nebenkern in spermatogenen Zellen und die Spermatogenese bei den Farnkräutern. Ib. **15**: 337–339.

——— 1898. Über die Cilienbildner in den spermatogenen Zellen. Ib. **15**: 140–144.

——— 1899. Über die Centrosome in den spermatogenen Zellen. Ib. **17**: 199–205.

BĚLAŘ, K. 1921. Protozoenstudien III. Arch. f. Protistenk. **43**: 431–462.

——— 1927. Beiträge zur Kenntnis des Mechanismus der indirekten Kernteilung. Naturwiss. **36**: 725–733.

BLACK, C.A. 1913. The morphology of *Riccia Frostii* AUST. Ann. of Bot. **27**: 511–532.

BLACKMAN, V.H. 1904. On the fertilization, alternation of generations, and the general cytology of the Uredineae. Ib. **18**: 321–373.

BOLLETER, E. 1905. *Fegatella conica* (L.) CORDA. Eine morphologisch-physiologische Monographie. Beih. z. bot. Centralbl. **18**: 327–408.

BROOKS, F.T. 1910. The development of *Gnomonia erythrostoma*, PERS. The cherry-leaf-scorch disease. Ann. of Bot **24**: 585–605.

CALDWELL, O.W. 1907. *Microcycas calocoma*. Bot. Gaz. **44**: 118–141.

CARRUTHERS, C. 1911. Contributions to the cytology of *Helvella crispa* FRIES. Ann. of Bot. **25**: 243–252.

CHAMBERLAIN, C. J. 1903. Mitosis in *Pellia*. Decennial Publ. Univ. Chicago **10**: 239–345.

——— 1909. Spermatogenesis in *Dioon edule*. Bot. Gaz. **47**: 215–236.

CONARD, A. 1939. Sur le mécanisme de la division cellulaire et sur les bases de la cytologie. Bruxelles.

DAVIS, B.M. 1898. Kernteilung in der Tetrasporenmutterzelle bei *Corallina officinalis* L. Ber. Deut. bot. Gesells. **16**: 266–272.

——— 1908. Spore formation in *Derbesia*. Ann. of Bot. **22**: 1–20.

DOBELL, C. 1908. The structure and life-history of *Copromonas*. Quart. Jour. mic. Sci. **52**: 205. (After WILSON, E.B. 1928)

DODGE, B.O. 1937. Spindle orientation and spore delimitation in *Gelasinospora tetrasperma*. Cytologia, FUJII jub. Vol.: 877–887.

DOFLEIN, F.J. 1918. Beiträge zur Kenntnis von Bau und Teilung der Protozoenkerne. 1. Die Kernteilung von *Polytomella agilis* AR. 2. Die Zell- und Kernteilung bei *Ochromonas granularis* n. sp. Zool. Anz. **49**: 289–306.

DRACINSCHI, M. 1932. Über die reifen Spermatozoiden bei den Pteridophyten (*Selaginella, Equisetum, Isoetes,* Filicales leptosporangiatae). Bull. Fac. Ştiinte Cernăuti **6**: 65–134.

EMBERGER, L. 1922. Evolution des plastes dans le règne végétal. Rev. Sci. **60**: 46.

ENTZ, G. 1918. Über die mitotische Teilung von *Polytoma uvella*. Arch. f. Protistenk. **138**: 324–354.

ESCOYEZ, E. 1907. Blépharoplaste et centrosome dans le *Marchantia polymorpha*. Cellule **24**: 247–256.

FARMER, J.B. 1910. On the cytological features exhibited by certain hybrid ferns. Ann. of Bot. **24**: 191–212.

FRASER, H.C.I. and BROOKS, W.E.St.J. 1909. Further studies on the cytology of the ascus. Ib. **23**: 537–549.

FUJII, K. 1930. Cytology in the present and past. (Japanese). Iwanami-Lecture, Biology. Tokyo.

——— and YASUI, K. 1933. On the mechanism of cell division and nuclear division II. The critical consideration on the various cell structures during cell and nuclear division. (Japanese) Found. for Prom. of Sci. Res. and Undertak. (Gakuzyutu-Kenkyû-Syôrei-Sikin oyobi Zigyô-Hôkoku) 1933, 179–184.

FULLMER, E.L. 1898. Cell division in pine seedlings. Bot. Gaz. **26**: 239–246.

GEITLER, L. 1934. Grundriss der Cytologie. Berlin.

——— 1918. Vergleichende Untersuchungen ueber den feineren Kern- und Chromosomenbau der Cladophoraceen. Planta **25**: 530–578.

GRAHAM, M. 1918. Centrosomes in fertilization stages of *Preissia quadrata* (SCOP.) NEES. Ann. of Bot. **32**: 415–420.

GUIGNARD, L. 1898. Centrosomes in plants. Bot. Gaz. **25**: 158–164.

HEITZ, E. 1931. Die Ursache der gesetzmässigen Zahl, Lage, Form und Grösse pflanzlicher Nukleolen. Planta **12**: 775–844.

——— 1932. a. Nukleolen und Chromosomen in der Gattung *Vicia*. Ib. **15**: 496–505.

——— 1932 b. Die Herkunft der Chromosomen. Dritter Beitrag zur Kenntnis der Beziehungen zwischen Kernstrukturen und qualitative Verschiedenheit der Chromosomen in ihrer Längsrichtung. Ib. **18**: 572–636.

HENNEGUY, L.E. 1898. Sur les rapports des cils vibratiles avec les centrosomes. Arch. Anat. Mic. **1**: 481–496.

HIRASE, S. 1894. Notes on the attraction spheres in the pollen cells of *Ginkgo biloba*. Bot. Mag. (Tôkyô) **8**: (359)–(364).

——— 1898. Études sur la fécondation et l'embryogénie du *Ginkgo biloba*. Jour. Coll. Sci. Imp. Univ. Tôkyô. **12**: 103–149.

HUMPHREY, H.B. 1906. The development of *Fossombronia longiseta*, AUST. Ann. of Bot. **20**: 83–108.

HUMPHREY, L. M. 1934. The meiotic divisions of haploid, diploid and tetraploid tomato with special reference to the prophase. Cytologia **5**: 278–300.

IKENO, S. 1898. Untersuchungen ueber die Entwicklung der Geschlechtsorgane und den Vorgang der Befruchtung bei *Cycas revoluta*. Jour. Coll. Sci. Imp. Univ. Tokyo **22**: 151–214.

IKENG, S. 1903. a. Beiträge zur Kenntnis der pflanzlichen Spermatogenese. Die Spermatogenese von *Marchantia polymorpha*. Beih. z. bot. Centralbl. **15**: 65–88.

——— 1903. b. The blepharoplast in the plant kingdom. (Japanese). Bot. Mag. (Tôkyô) **36**: (278)–(290).

——— 1906. Zur Frage nach der Homologie der Blepharoplasten. Flora **96**: 538–542.

JAHN, E. 1904. Myxomycetenstudien III. Kernteilung und Geisselbildung bei den Schwärmern von *Stemonitis flaccida* LIST. Ber. Deut. bot. Gesells **22**: 84–92.

KOFORD, C. A. and SWEZY, O. 1915. Mitosis in *Trichomonas*. Proc. Nat. Acad. Sci. **1**: 315.

KRETSCHMER, H. 1930. Beiträge zur Cytologie von *Oedogonium*. Arch. f. Protistenk. **71**: 101–138.

KUCZYNSKI, M. H. 918. Über die Teilung der *Trypanosomen*zelle, etc. Arch. f. Protistenk. **38**: 94–112.

KUSANO, S. 1930. Cytology of *Synchytrium fulgens*. SCHRÖT. Jour. Coll. Agr. Imp. Univ. Tôkyô **10**: 347–388.

KUWADA, Y. 1930 Karyology. (Japanese) Iwanami-Lecture, Biology. Tôkyô.

——— 1937. The structure of chromosome. (Japanese) Tôkyô.

——— and SUGIMOTO, T. 1928. On the staining reactions of chromosomes. Protoplasma **3**: 531–535.

LACKEY, J. B. 1933. Studies in the histories of Euglenida III. The morphology of *Peranema trichophorum* EHRENS. with special reference to its kinetic elements and the classification of the Heteronemidae. Biol. Bull. **65**: 238–248.

LANG, A.G. 1936. Spermatogenesis in *Marsilia*. Jour. Elisha Mitchell Sci. Soc. **52**: 307–336.

LAWSON, A.A. 1909. The gametophytes and embryo of *Pseudotsuga Douglasii*. Ann. of Bot. **23**: 163–180.

LEWIS, J.F. 1909. The life history of *Griffithsia Bornetiana*. Ib. **23**: 639–690.

MASACRÉ, M. et PICARD, P. 1933. Action de l'éther et du chloroforme sur la structure de la cellule végétale (racines *d'Allium sativum* L.). Bull. Soc. Bot. France **80**: 326–333.

MATSUURA, H. 1935. On the relation between the nucleolus and chromosome. (Japanese). Bot. and Zool. **3**: 1589–1594.

——— and GONDO, A. 1935. A karyological study on *Peziza subumbrina* BOUD., with special reference to a heteromorphic pair of chromosomes. Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. V, Vol. **III**, No. 5: 205–217.

MIYAKE, K. 1905. On the centrosome of Hepaticae. Bot. Mag. (Tôkyô) **19**: 98–101.

MOTTE, J. 1928. Contribution à la connaissance cytologique des Muscinées. Ann. Sci. Nat. Bot. X, **10**: 298–543.

MOTTIER, D. M. 1904. The development of the spermatozoid of *Chara*. Ann. of Bot. **18**: 245–254.

MÜCKE, M. 1908. Zur Kenntnis der Eientwicklung und Befruchtung von *Achlya polyandra* de BARY. Ber. Deut. bot. Gesells. **26**: 367–378.

OVERTON, J.B. 1909. On the organization of the nuclei in the pollen mother cells of certain plants, with special reference to the permanence of the chromosomes. Ann. of Bot. **23**: 19–61.

PEKAREK, J. 1932. Ein vergessenes Objekt für das Stadium der Kern- und Zellteilungsvorgänge in Leben. Planta **16**: 788–800.

PICKARSKI, G. 1937. Cytologische Untersuchungen an *Paratyphus* und Colibakterien. Arch. f. Protistenk. **8**: 428–439.

PRISCILLA, E.F. and BROWN, R.H. 1930. Nuclear behaviour in the mitosis of plant cells. Quart. Jour. mic. Sci. **73**: 197–214.

PROWAZEK, S. 1904. Untersuchungen über einige parasitische Flagellaten. Arb. a.d. Kais. Gesundheitsamt. **21**. (After WILSON, E.B. 1928)

REICH, K. 1926. Beiträge zur Entwicklungsgeschichte der Protophyten. I. Zur Kenntnis der Entwicklungsgeschichte und Cytologie von *Stigeoclonium*. Arch. f. Protistenk. **53**: 435–458.

SAKAMURA, T. 1927. Chromosomenforschung an frischem Material. Protoplasma **1**: 537–565.

SASAKI, M. 1935. Spermatoteleosis in *Nitella* with special reference to the morphology of the blepharoplast. Bot. Mag. (Tôkyô) **49**: 513–519.

SATÔ, D. 1936. The recent studies on the nucleolus-chromosomes in plants I, II. (Japanese) Bot. and Zool. **5**: 817–824, 991–996.

SCHAEDE, R. 1929. Uber das Verhalten des Nucleolus während der Kernteilung. Protoplasma **5**: 41–54.

SCHAFFNER, J.H. 1898. Karyokinesis in the root tips of *Allium Cepa*. Bot. Gaz. **26**: 225–238.

——— 1908. The centrosomes of *Marchantia polymorpha*. Ohio Nat. **9**: 383–388.

——— 1909. The reduction division in the microsporocytes of *Agave virginiana*. Bot. Gaz. **47**: 198–214.

SCHUSSNIG, B. 1930. Die mitotische Kernteilung bei *Ulothrix zonata* KUETZING. Zeit. Zellf. mik. Anat. **10**: 642–650.

——— 1938. Vergleichende Morphologie der niederen Pflanzen. Erster Teil: Formbildung. Berlin.

SELIM, A.G. 1931 A cytological study of *Oryza sativa* L. Cytologia **2**: 1–26.

SENJANINOVA, M. 1926. Das Verhalten des Nucleolus und der Trabanten während der somatischen Mitosen und der Reifteilungen bei *Ranunculus acer* L. Zeit. Zellf. mik. Anat. **3**: 417–430.

SHARP, L.W. 1912. Spermatogenesis in *Equisetum*. Bot. Gaz. **54**: 89–119.

——— 1914. Spermatogenesis in *Marsilia*. Ib. **58**: 419–431.

——— 1920. Spermatogenesis in *Blasia*. Ib. **69**: 258–268.

——— 1934. Introduction to cytology. New York and London.

SHAW, W.R. 1898. Über die Blepharoplasten bei *Onoclea* und *Marsilia*. Ber. Deut. bot. Gesells. **16**: 177–184.

SHINKE, N. and SHIGENAGA, M. 1933. A histochemical study of plant nuclei in rest and mitosis. Cytologia **4**: 189–221.

SHOWALTER, A.M. 1928. Studies in the cytology of Anacrogyne V. Hybrid fertilization in *Riccardia pinguis*. Cellule **38**: 295–348.

SINOTÔ, Y. 1930. Cytology in Japan, present and past. (Japanese). Iwanami-Lecture. Tôkyô.

SOROKIN, H. 1927. A study of meiosis in *Ranunculus aceris*. Amer. Jour. Bot. **14**: 76–84.

——— 1929. Idiograms, nucleoli and satellites of certain Ranunculaceae. Ib. **16**: 407–420.

STRASBURGER, E. 1892. Schwärmsporen, Gameten, pflanzliche Spermatozoiden und das Wesen der Befruchtung. Hist. Beitr. **4**: 49–158.

SUEMATSU, S. 1936. Studies on the nucleolus of plant cell. I, II, III. (Collective review) Bot. and Zool. **5**: 1876–1881, 2042–2048, 2217–2223.

SWINGLE, W.T. 1897. Zur Kenntnis der Kern- und Zellteilung bei den Sphacelariaceen. Jahrb. f. wiss. Bot. **30**: 297–350.

TANAKA, N. 1939. Chromosome studies in Cyperaceae V. Pollen development of *Carex grallatoria* MAXIM. var. *heteroclita* KÜKENTH. ex MATSUM. Jap. Jour. Gen. **15**: 153–157.

TROW, A.H. 1904. On fertilization in the Saprolegniaceae. Ann. of Bot. **18**: 541–569.

VAN LEEUWEN-REIJNVAAN, J.W. 1908. Über die Spermatogenese der Moose, speciell mit Berücksichtigung der Zentrosomen- und Reduktionsteilungen. Ber. Deut. bot. Gesells. **26**: 301–309.

VON DERSCHAU, M. 1904. Wanderung nukleolarer Substanz während der Karyokinese und in lokal sich verdickenden Zellen. Ber. Deut. bot. Gesells. **22**: 440–411.

VON STOSCH, H.A. 1937. Untersuchungen über die Sexualität und Apogamie bei Didymiaceen. Planta **23**: 623–656.

WADA, B. 1932. Mikrurgische Untersuchungen lebender Zellen in der Teilung I. Die Bildung von tetraploidkernen, zweikernigen Zellen und anderen abnormen Teilungsbildern. Cytologia **4**: 114–134.

——— 1935. Ditto II. Das Verhalten der Spindelfigur und einige ihrer physikalischen Eigenschaften in den somatischen Zellen. Ib. **7**: 198–213.

WAGER, H. 1904. The nucleolus and nuclear division in the root-apex of *Phaseolus*. Ann. of Bot. **18**: 29–55.

WALKER, N. 1913. On abnormal cell-fusion in the archegonium; and on spermatogenesis in *Polytrichum*. Ib. **27**: 115–132.

WEBBER, H.J. 1897. Notes on the fecundation of *Zamia* and the pollen tube apparatus of *Ginkgo*. Bot. Gaz. **24**: 225–235.

——— 1901. Spermatogenesis and fecundation of *Zamia*. U. S. Dept. Agr. Bur. Pl. Ind. Bull. **2**: 1–100.

WELSFORD, E.J. 1908. Further contribution to the cytology of the Ascomycetes. Ann. of Bot. **22**: 465–477.

WILLIAMS, J.L. 1904. Studies in the Dyctyotaceae. I. The cytology of the tetrasporangium and the germinating tetraspore. Ib. **69**: 141–160.

——— 1904. Ditto. II. The cytology of the gametophyte generation. Ib. **18**: 183–204.

WILSON, E.B. 1928. The cell in development and heredity. New York.

WILSON, M. 1909. On spore formation and nuclear division in *Mnium hornum*. Ann. of Bot. **23**: 141–157.

——— 1910. Preliminary note on the spermatogenesis of *Mnium hornum*. Ib. **24**: 235.

——— 1911. Spermatogenesis in the Bryophyta. Ib. **25**: 415–457.

WILSON, R.S. 1898. Bot. Gaz. **26**: 448–449.

WOODBURN, W.L. 1911. Spermatogenesis in certain Hepaticae. Ann. of Bot. **25**: 299–313.

——— 1913. Spermatogenesis in *Blasia pusilla*. Ib. **27**: 93–101.

——— 1915. Spermatogenesis in *Mnium affine* var. *ciliaris* (GREV.), C. M. Ib. **29**: 441–456.

YAMAHA, G. 1927. Experimentelle zytologische Beiträge I. Mitt. Orientierungsversuche an den Wurzelspitzen einiger Pflanzen. Jour. Fac. Sci. Imp. Univ. Tôkyô **2**: 1–214.

——— 1932. General cytology. (Japanese) Tôkyô.

——— and SINOTÔ, Y. 1925. On the behaviour of the nucleolus in the somatic mitosis of higher plants, with microchemical notes. Bot. Mag. (Tôkyô) **39**: 205–219.

——— und SUEMATSU, S. 1936. Beiträge zur Kenntnis pflanzlicher Nukleolen. Sci. Rep. Tôkyô-Bunrika-Daigaku, Sec. B, **3**: 21–34.

YAMANOUCHI, S. 1908. Spermatogenesis, oogenesis and fertilization in *Nephrodium*. Bot. Gaz. **45**: 145–175.

——— 1909. Mitosis in *Fucus*. Ib. **47**: 173–197.

YAZAWA, H. 1931. On the spermatogenesis in *Makinoa crispa*, (ST.) MIYAKE. Cytologia **2**: 157–173.

YUASA, A. 1934. Studies in the cytology of reproductive cells I. On the planocytes in five forms of Myxomycetes. Bot. Mag. (Tôkyô) **48**: 720–729.

——— 1935. a. Ditto. III. The genesis of the flagellum in the planocyte of *Fuligo septica* GMELIN. Ib. **49**: 538–545.

——— 1935. b. Ditto. IV FEULGEN's nucleal-reaction applied to the blepharoplast. Ib. **49**: 868–875.

——— 1936. *a*. Cytomorphological study of *Rhodospirillum longum* HAMA. Ib. **50**: 93–98.

——— 1936. *b*. Studies in the cytology of Pteridophyta IX. On the nature of the border-brim. Ib. **50**: 151–158.

——— 1936. *c*. Centrosome and blepharoplast in *Stemonitis*. (Japanese). Ib. **50**: (5)–(6).

——— 1936. d. FEULGEN's nucleal staining applied to Pteridophyta. Proc. Imp. Acad. Tôkyô **12**: 266–268.

——— 1937. *a*. Studies in the cytology of Pteridophyta XIV. Spermatoteleosis and fertilization in some ferns, with special reference to border-brim. Jap. Jour. Bot. **5**: 17–35.

——— 1937. *b*. Studies in the cytology of reproductive cells V. Centrosome and blepharoplast in Myxomycetes. Bot. Mag. (Tôkyô), 51, SHIBATA Com. Art. :332–337.

——— 1938. a. The stainability of blepharoplast. Kwagaku **8**: 53.

——— 1938. *b*. Studies in the cytology of Pteridophyta XV. A critical consideration of cytological fixation and staining in the sporophytic cells, prothallium cells and spermatozoids of *Dryopteris uniformis* MAKINO. Jap. Jour. Bot. **9**: 145–191.

——— 1938. *c*. Cytological studies of spermatoteleosis and fertilization in Pteridophyta, with special reference to the border-brim. Stud. Tokugawa Inst. **4**: 1–116.

——— 1938. *d*. Critical considerations on the origin of blepharoplast.—The nucleolar substance theory. (Japanese) Bot. Mag. (Tôkyô) **52**: 318–328.

ZIRKLE, C. 1931. Nucleoli of the root-tip and cambium of *Pinus Strobus*. Cytologia **2**: 85–105.

Some anatomical notes on *Cycas revoluta*, especially on its anomalous secondary growth[1]

By Tsugio HANDA

With plate III and 18 text-figures

(Received August 30, 1939)

The family Cycadaceae has been repeatedly studied by many anatomists because of its phylogenetic importance, and as regards the anomalous secondary growth in this family it is well known that the stem of the genera *Cycas, Macrozamia* and *Encephalartos* develops successive rings of wood and bast. Though most of the investigations on this anomaly have been restricted to the stem, such an anomalous structure is also found in the root. I tried in this study to trace the anomalous structure throughout the plant body in *Cycas revoluta*, and, in addition, paid attention to certain interesting anatomical points, such as the leaf trace or the cortical concentric bundles.

The material was obtained from Okinawa Island, one of the Riu-kiu Islands, where *C. revoluta* is growing in abundance. The opportunity for collecting the material I owed to the goodwill of Prof. Y. OGURA, under the guidance of whom this investigation was carried out. It is a great pleasure for me to express here my hearty thanks to him. Among the materials the seedlings and the plants raised from adventitious buds were made available for this investigation by the kindness of Prof. T. NAITO of the Kagoshima Agricultural College and of Mr. K. MIYAI of the Kagoshima-shiritsu Joshi Kogyo Gakko. I take this opportunity to express my deepest thanks to them.

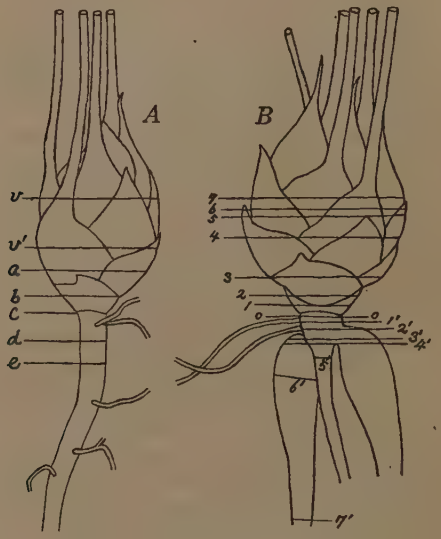
Anomalous secondary growth

GREGG (1887) observed an anomalous structure in a certain region of the tap root of a seedling in *Cycas Seemanni*, while WORSDELL (1898) found in the hypocotyl of the seedling of *Cycas revoluta* several concentric

(1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 230.

strands lying outside the central cylinder and thought that these strands represented an early stage of the extrafascicular rings clearly shown in thickened stems. My material proved that WORSDELL was quite mistaken in his interpretation of the nature of these concentric bundles.

On the other hand I aimed at making it clear how the transition of the successive rings from the stem to the root could occur. Generally in adult plants vigorous adventitious roots appear in the basal region of the stem and one can scarcely tell the primary root from these adventitious ones. Such a condition makes it difficult to trace the structural connections between the stem and the root. However the seedlings of this plant are very suitable for this purpose, as they show a well-developed tap root.



Text-fig. 1. Three-year-old seedlings. A, seedling no. 1; B, seedling no. 2. both ×ca. 2/3.

1) *The seedling.* Five seedlings, all three years old, were cut transversely or stripped down to skeletons. The descriptions, for convenience's sake, will be chiefly based on the actual structure shown in two of the seedlings. One (seedling no. 1) was about 3 cm. thick, and the other (seedling no. 2) about 4 cm. thick in the thickest part of the stem (text-fig. 1 and pl. III, figs. 1–2).

Seedling no. 1, in order to follow the structural transition from the stem to the root, is cut transversely at different levels of the plant body. 21 main sections are shown in successive order in text-fig. 2; section 1

is made at the level *a* and section 21 at the level *e* (cf. text-fig. 1, A). Sections 5–7 are cut through the cotyledonary region (containing the level *b*), and section 16 through the level *c*, which in appearance is the border between the stem and the root. Just beneath the cotyledonary

Text-fig. 2. Cross sections cut at different levels of seedling no. 1. *n*, normal ring; *s*, first secondary ring; *m*, middle ring; *c*, concentric bundle. ×ca. 6.

region, viz. in section 8 we see three vascular rings, the inner, the middle and the outer. The inner is the normal vascular ring. The outer is the first secondary ring, but this is still very thin and is not found below beyond the level *c*. The middle ring has the appearance of being the

first secondary ring, and this is why WORSDELL was led to a misinterpretation. In fact, the middle ring is quite independent from the successive development of the vascular rings, and the middle and the outer ring each belong to different categories of anomaly. It is certain that the middle ring develops earlier than the outer, or first secondary ring; and the middle ring has intimate structural relations with the inner, or normal vascular ring. The middle ring shows normal orientation of xylem and phloem. In section 8 this ring is seen in a well developed stage. Higher up, the ring splits into smaller segments or strands, and becomes joined to the central cylinder. In sections 3–4, segments of the middle ring merge into the central cylinder, and still higher up we cannot find any traces of this anomalous structure (sections 1–2).

Tracing downwards the structure shown in section 8, we find that the central cylinder passes very soon into the root structure, two primary xylem groups standing opposite to each other on the cotyledonary sides. Section 10 is definitely of root structure and even section 9 may be regarded as of root structure. In other words, the transition from the stem to the root occurs just beneath the cotyledonary region and not at the level *c*, which in appearance is the border between the two parts.

The middle ring shown in section 8 grows smaller, as it goes downward; some of the segments of the ring join on the cotyledonary sides to the central cylinder (sections 10–13), while the remaining segments gradually become concentric (sections 9–13). These concentric bundles are situated on the intercotyledonary sides. In section 13, two typically concentric bundles are seen; one on the left hand disappears down in section 18, and the other on the right hand becomes scarcely discernible in section 20. Besides these two bundles, there appears a small concentric bundle in section 14, and furthermore another small collateral one in section 15. These small bundles soon disappear, too. And no bundles relating to the anomalous structure are found in section 21 or at the level *e*.

The nature of the mother tissue of this anomalous structure was not definitely determined, as it was impossible in my material to distinguish between the endodermis and its neighbouring tissues. However, for a certain reason it seems that the structure in question originates in the pericyclic layer, and this is also thought to be true in view of what was said by WORSDELL, who observed concentric strands within a well-marked pericyclic layer in the seedling of *Cycas revoluta*.

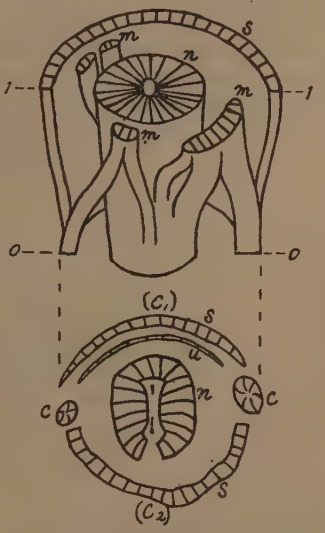
Next we turn to the anomaly represented by the successive development of wood and bast. In seedling no. 1, as above-mentioned, the first secondary ring is found, but this is still very thin. The ring is comparatively thick in the cotyledonary region. The segments composing the ring are broken at higher levels into smaller ones; and the ring is repre-

sented only by one or two small strands at the level *v'* (text-fig. 1, A), where the central cylinder is about 0.6 mm. in thickness. Higher above this level the secondary ring is not found at all.

Going down from the cotyledonary region, the secondary ring grows gradually thinner and disappears completely at the base of the tap root. However, the ring sooner or later will extend into the tap root, as is really the case in seedling no. 2 which will be dealt with later on.

In the seedlings of this age the central cylinder grows larger progressively by the active division of its cambium. The cambium of the secondary ring is also dividing. Therefore, the view presented by certain authors that in *Cycas* as well as in *Macrozamia* and *Encephalartos*, the secondary cambium appears after the primary cambium has lost its function is not applicable in the present species.

The question where the successive rings of wood and bast originate in the polyxylic stem of *Cycas* has been disputed by many authors. One group of authors claims that the rings arise in the pericycle within the well-marked endodermis, while another group claims that they arise in the cortex. These opposing opinions are not based upon observations made in the same species, but on those made in different species, and both may prove to be true, if a wider range of observations is made in polyxylic cycads.

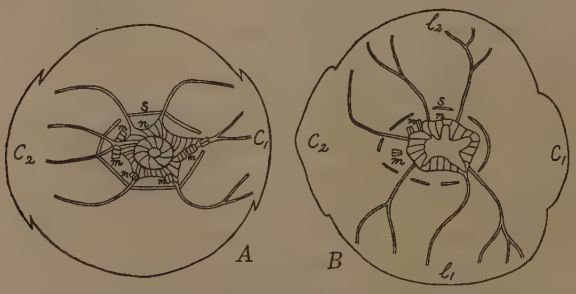


Text-fig. 3. Vascular skeleton of the transition region of seedling no. 2. *n*, normal ring; *s*, first secondary ring; *m*, middle ring; *c*, concentric bundle; *u*, supernumerary ring; (c_1) and (c_2), cotyledonary sides; *0* and *1*, levels shown in text-fig. 1, B. ×ca. 6.

As regards my seedlings, the endodermal layer is not found in the stem. It is also impossible to distinguish the endodermis in the upper part of the tap root; while in the lower part, where the first secondary ring is not yet differentiated, the endodermal layer is shown clearly. Such being the case, it cannot be directly ascertained where the first secondary ring originates. However, after making comparative observations, I lean towards the idea that the mother layer of the successive secondary rings is in the pericycle.

The general features of the anomalies were just described in the case of seedling no. 1, but it seems necessary to go into further details.

For this purpose seedling no. 2 was used. This seedling has two tuberous roots at the basal part of the tap root and shows some modifications in its anomalous structure. This point will also be touched on. Text-fig. 1, B presents seedling no. 2. In this seedling the part between the levels *0* and *1* is stripped to a skeleton and the vascular skeleton thus made is shown semidiagrammatically in text-fig. 3, in which the transverse section at the level *0* is also exhibited. In this section (c_1) and (c_2) indicate the cotyledonary sides; on the side (c_1) there is found a very thin fibrovascular ring (segment) inside the first secondary ring. This supernumerary ring on the side (c_1) and a part of the secondary ring on the side (c_2) are omitted in the diagram. At the level *1* the middle ring, as named above in the description of seedling no. 1, is clearly seen between the central cylinder and the first secondary ring. Lower



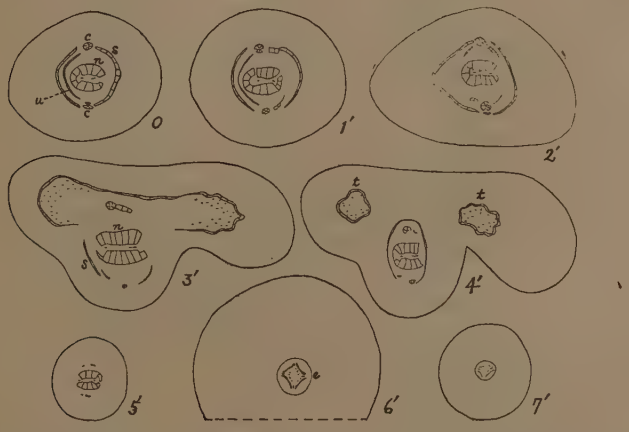
Text-fig. 4. Thick sections from seedling no. 2, as seen from the lower side of the sections. c_1 and c_2, cotyledons; l_1 and l_2, first and second leaves. Further explanation in the text. ×2.

down, segments of the middle ring split into smaller parts, of which some pass inwards and join the central cylinder on the cotyledonary sides, while the rest pass outwards to form concentric bundles on the intercotyledonary sides. At the level *0* (cf. the cross section) one concentric bundle of such nature is seen on each of the intercotyledonary sides.

In order to trace the middle ring upwards from the level *1* the seedling was cut at the levels *1*, *2*, and *3*, and the resulting two thick sections were skeletonized. These two diagrammatic skeletons are presented in text-fig. 4, as seen from the lower side; A is a skeleton stripped from the section *1–2* and B from the section *2–3*. In A, a hexagonal cotyledonary plate is seen, the corners of which taper into cotyledonary traces. Most of the segments of the middle ring become attached to the lower side of the cotyledonary plate, which occurs usually at the bases of the

cotyledonary traces. The rest of the segments pass further upwards but soon fuse to the central cylinder (text-fig. 4, B). Now it is clear that the middle ring is quite independent in development from the successive secondary rings of wood and bast.

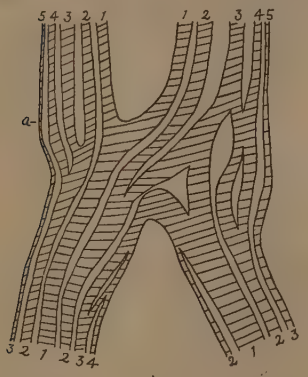
As the first secondary ring is differentiated in the tap root in seedling no. 2, which is not yet the case in seedling no. 1, it is possible to trace this ring, together with the concentric bundles, down into the tap root. Sections in text-fig. 5 are cut through seedling no. 2 at the levels indicated in text-fig. 1, B. Two concentric bundles shown at the level *0* (section 0) persist down through the tap root, and their lower parts are still found at the level *5'* (section 5'), where the first secondary



Text-fig. 5. Cross sections of seedling no. 2, made at different levels indicated in text-fig. 1, B. *n*, normal ring; *s*, first secondary ring; *c*, concentric bundle; *u*, supernumerary ring; *t*, central cylinder of tuberous root; *e*, endodermis. ×2.

ring is not found at all. In section 3' two tuberous roots are just departing. Here the first secondary ring divides into three parts; one remains as the first secondary ring of the tap root, while the rest become the central cylinders of the tuberous roots. These central cylinders are completed in section 4'. Besides, it is noticed that there appear scattered tracheids in the medullary part of the tuberous roots. Such scattered tracheids are present throughout the length of the tuberous roots (sections 6' and 7'). It is interesting to note that the central cylinders of the tuberous roots have a direct connection with the first secondary ring of the tap root, but not with the central cylinder of the latter.

Again, turning to section 0, there is found a very thin vascular band on the left hand of the central cylinder, inside the first secondary ring. It is certain that this band is of late formation. The band dwindles both upwards and downwards as it goes far from the level *0*, and in the downward direction disappears at the level *2'*. The band is growing thick by means of cambium and it does not seem impossible that it will later extend all around the central cylinder so as to form a complete ring.



Text-fig. 6. Median longitudinal section of the transition region of a 17 cm. thick plant, showing vascular connections between the stem and the roots. At the level *a*, medullary part of the stem disappears. *1*, normal ring; *2*, *3*, *4*, successive secondary rings. ×1/3.

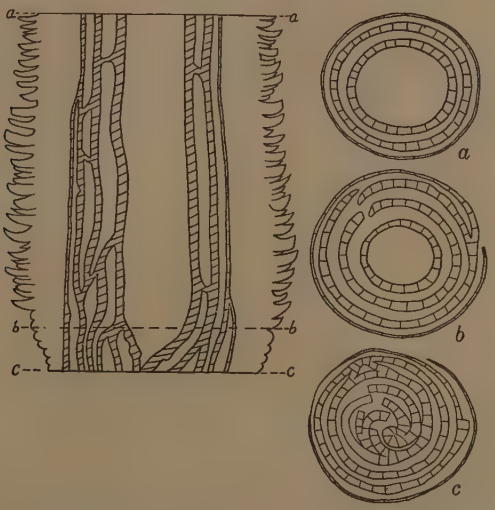
2) *The large plant.* The vascular connections between the stem and the root will be briefly considered in two large plants. It is not known whether these plants originated from the seeds or from the adventitious buds. One plant is 53 cm. long, and 17 cm. thick in the middle part of the stem, and the lower end of its stem passes into three roots of nearly equal thickness. Text-fig. 6 is a longitudinal section through the transition region of this plant, in the figure only the xylem part of the rings being displayed, while the stem and two roots are cut nearly in their median planes. In this figure five rings are seen in the stem and three or four in the roots, and the fifth ring of the stem is continuous with the third ring of the roots. And higher up, half-way along the stem only two vascular rings are found.

As is clear from the above-mentioned facts, cross sections at different heights of the stem will show a different number of vascular rings. The other of the two large plants is about 1 meter high; in text-fig. 7 there are shown a longitudinal section through the basal region of the stem and three transverse sections cut at different levels of the same stem. At the level *a* there are found three rings—two secondary rings in addition to the normal one. These three rings persist up the stem; but as they go down the stem, they become complicated, and there are found as many as six rings at the level *c*, which is regarded as the very base of the stem. Such complexity of the vascular structure at the stem base varies according to different individuals, depending on the number of the roots produced or on the time when the roots appear. The principle

of the structural relations between the stem and the root will be considered below in younger plants originating from adventitious buds.

3) *The plant produced asexually from the adventitious bud.* Two asexually produced plants came to my hand, one 15 cm. and the other 10 cm. thick in the middle part of the stem, both of which were separated probably a few years before from their mother plants. The stem of them is not yet sufficiently elongated but presents an egg-like appearance. However the first secondary ring is extending up to the middle height of the stem. Vigorous adventitious roots appear at the base of the stem.

In the larger of the two plants the central cylinder of the stem



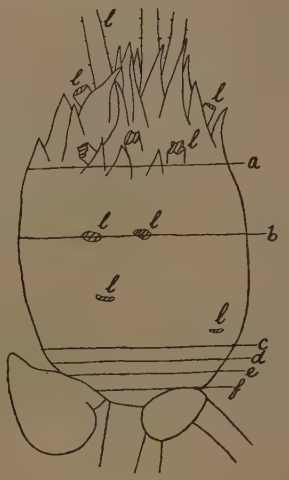
Text-fig. 7. Median longitudinal section through the lower part of a 18 cm. thick plant, and the cross sections at the levels indicated. ×1/4.

divides into a few parts in the basal region of the stem, and each of the parts passes into a root so as to form there two secondary rings, the first and the second, as well as the central cylinder. This structural complexity results on the other hand in closing the central cylinder of the stem in the basal region of the latter. Text-fig. 8 is a longitudinal section of this plant, the stem and one of the adventitious roots being cut nearly in their median planes. It may be seen from the figure that the normal (*1*) and the secondary rings (*2, 3*) of the root are together equivalent to a part of the normal ring of the stem. Outside these rings a new vascular ring (*4*) is seen, which passes upwards to form the first secondary ring of the stem, while it dwindles and dis-

appears a short distance below the stem base. Later this ring will extend further down the root and form the third secondary ring of the root. It is noticed in text-fig. 8, that a layer of scattered tracheids is differentiated just inside the central cylinder at the basal region of the stem. The same is also the case in the other, smaller plants.

The smaller of the two plants (text-fig. 9) has three thick adventitious roots at the base of the stem. Besides there are found two adventitious buds at the stem base. The vascular relations between the stem

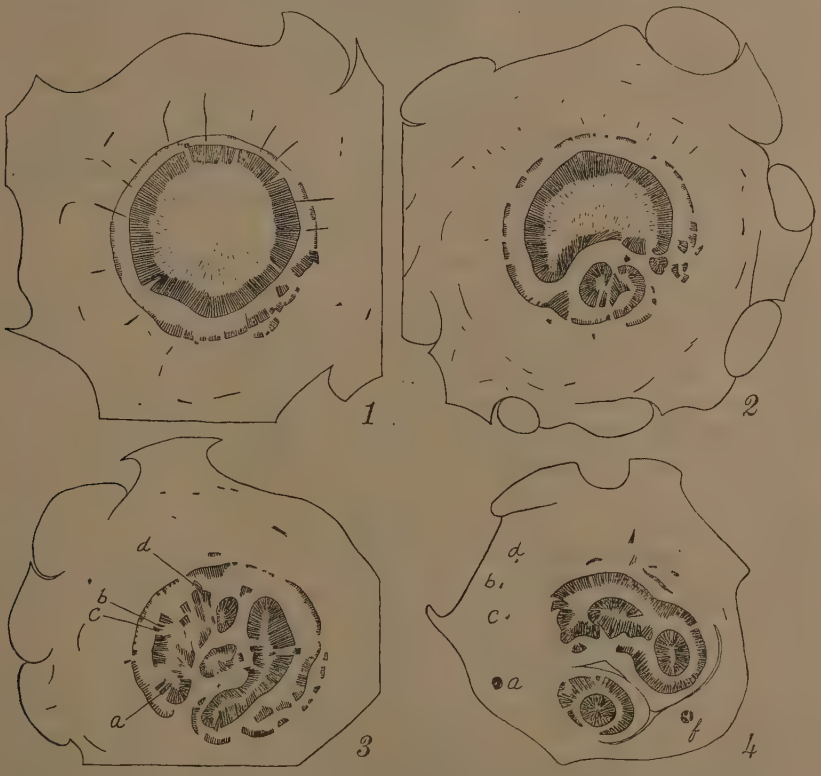
Text-fig. 8. Median longitudinal section through the larger of the two asexually produced plants, showing vascular connections between the stem and the roots. *1*, normal ring; *2*, *3*, *4*, successive secondary rings; *f*, foliage leaf. ×1/2.



Text-fig. 9. The smaller of the two asexually produced plants. Three adventitious roots and two adventitious buds are seen at the stem base. *l*, foliage leaf. ×1/3.

and the roots are the same in principle as those observed in the larger plant mentioned above. At the level *c* (indicated in text-fig. 9) there are seen two vascular rings, the central and the first secondary ring (text-fig. 10, 1). A little below, at the level *d*, the central cylinder divides into two parts (text-fig. 10, 2). The smaller part soon enters one of the adventitious roots and there forms the normal and the first secondary ring. The larger part also, after becoming divided into two, forms the normal and the first secondary rings of two remaining roots.

This may be understood in text-fig. 10, 4, representing the section made at the level *f*. Thus the central cylinder of the stem is used up in the formation of the normal and the first secondary rings of the adventitious roots. The section of text-fig. 10, 3, is cut between the levels *d* and *f*, i. e. at the level *e*. Here the vascular system of the central cylinder is very complicated owing to the structural transition just mentioned.



Text-fig. 10. Cross sections of the plant shown in text-fig. 9. Sections 1, 2, 3, 4 are made at the levels *c*, *d*, *e*, *f* respectively. The bundles *a*-*d* enter the larger of the two adventitious buds, and the bundle *f* enters the smaller one. ×3/4.

In the above paragraphs the vascular structure of *Cycas revoluta* was described in the seedlings, large plants, and asexually produced plants. In the seedlings it is seen that the first secondary vascular ring extends up the stem and down the tap root. The cambium of the central cylinder and that of the first secondary ring are active at the

same time and it does not happen in this species that the cambium of the first secondary ring appears for the first time upon the dying out of the cambium activity of the central cylinder. Where the mother layer of the secondary cambium originates is a puzzling problem, but it is likely that the secondary cambium arises in the pericycle, in view of deductions made from the structure shown in a certain part of the root.

When older the plant will successively produce newer secondary rings in the same way as is observed in the case of the first secondary ring. However the vascular system will then be complicated at the basal region of the stem owing to the appearance of the adventitious roots. The structure of the large plants above-mentioned may present such a later stage, but we cannot lay stress on that structure, as it is not certain whether these plants arose from the seeds or from the adventitious buds.

The plants originating from an adventitious bud presents a peculiality in the formation of vascular rings at the base of the stem. When an adventitious bud becomes separated from its mother plant, the bud produces a few adventitious roots from its base; the central cylinder of the stem of the bud is divided at the stem base among the adventitious roots and each of the vascular parts thus divided forms two or three vascular rings of an adventitious root. The first secondary ring, in the true sense of the term, appears outside the vascular system just mentioned, and the differentiation of this ring extends from the stem base both upwards and downwards through the stem and the adventitious roots.

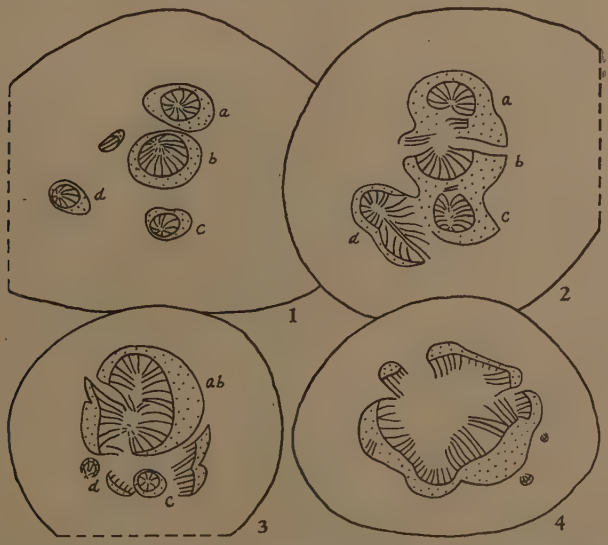
In one of the seedlings a supernumerary ring is being differentiated between the normal and the first secondary ring in the bordering region of the stem and the root. This will show that the parenchyma of this region easily returns to embryonal conditions. The complexity of the vascular system shown in the stem base of the large plants will be due partly to this kind of division of the parenchyma.

Another anomalous structure is present in the seedlings. Just a short distance below the cotyledonary plane a vascular ring lies between the normal and the first secondary ring. This ring is about as thick as the normal ring and of course far thicker than the first secondary ring; and a little higher up segments of this ring soon become added to the cotyledonary plate or to the central cylinder of the stem. In the opposite direction about one half of the segments join the central cylinder on the cotyledonary sides of the plant, while the other half become concentric and go down the tap root as the concentric bundles lying on the intercotyledonary sides. But these concentric bundles gradually become smaller as they go down, and disappear completely at a certain level, not very far from the stem base. It is thought that this anomalous structure is formed as early as the central cylinder, judg-

ing from its good development. Besides, it is clear from the above-mentioned that the structure in question is quite different from the system of the successive secondary rings. In my large plants, their origin unknown, nothing is found which corresponds to this anomaly. However, before considering the future of this anomalous structure it is necessary to make careful observations on adult plants, raised undoubtedly from seeds. But at any rate the present anomalous structure shown in the seedlings will be of a certain phylogenetic importance.

Vascular supply of the adventitious buds

The smaller of the two asexually produced plants above-mentioned has two adventitious buds at the basal region of the stem (text-fig. 9,

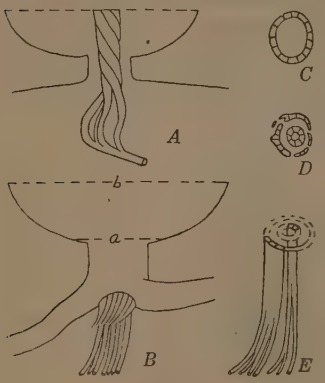


Text-fig. 11. Cross sections through the shaft of the larger adventitious bud of the plant shown in text-fig. 9. Bundles shown in text-fig. 10 may be followed in the shaft. Explanation in the text. ×5.

pl. III, fig. 3). In this plant the vascular connections between the adventitious buds and the mother plant were investigated. In text-fig. 10, 4, which represents a section through the basal part of this plant, a bundle is seen below in the right-hand corner of the section. This bundle is formed by the fusion of five small bundles of inverse orientation, which parted at higher levels from the vascular rings of the stem. Lower down,

the bundle passes outwards and enters the smaller adventitious bud so as to form the central cylinder of the latter.

On the left hand of the same section we see four small bundles *a*, *b*, *c*, *d*, all having been sent out from the vascular rings at slightly higher levels of the stem. In text-fig. 10, 3, the four bundles are seen just leaving the vascular rings. They take their course through the cortex towards the larger adventitious bud. These bundles are also of inverse orientation, and as they grow near the adventitious bud, they become concentric. The bud has a short shaft which connects the bud with the mother plant. At the entrance into this shaft the four bundles are quite concentric and take mutual positions as shown in text-fig. 11, 1. As the concentric bundles pass on through the shaft, they open and join together (text-fig. 11, 2 and 3) and at the upper end of the shaft form a single vascular cylinder (text-fig. 11, 4).



Text-fig. 12. Two adventitious buds produced on a plant. A, the smaller one. B–E, the larger one: C, cross section at the level *b*; D, cross section at the level *a*; E, diagram showing vascular connections between the bud and the mother plant. ×2/3.

The number of vascular bundles between the bud and its mother plant was observed also in two other adventitious buds, formed at the stem base of a large, male plant. One of the adventitious buds, 4.5 cm. thick, shows in its stem part only a single vascular cylinder, which lower down at the base of the shaft divides into four concentric bundles (text-fig. 12, A). These concentric bundles, passing into the cortex of the mother plant, soon unite together into a single concentric bundle; the further course of which in the mother plant was, to my regret, not traced.

The other adventitious bud, 55 cm. thick, has two adventitious roots at the base of the shaft, but still adheres to the mother plant by vascular connections (text-fig. 12, B). This bud shows two vascular rings at the level *a* (text-fig. 12, D), but higher up these rings join together into a single vascular ring. Text-fig. 12, C is a section at the level *b*. In the connecting region between the bud and the mother plant six concentric bundles are found to enter the shaft of the bud from the mother plant. Two of these form a part of the inner vascular ring and the rest form a part of the outer ring of the bud, as is shown in text-fig. 12, E. The remaining parts of the inner and outer rings are formed by the bundles passing from the adventitious roots. This active participation of the

adventitious roots in the constitution of the vascular rings will show that in this bud the adventitious roots arose very early in the process of formation of the bud.

Summarizing the above, it is possible to state that the number of bundles connecting an adventitious bud to its mother plant is not constant, and besides, in the vascular rings of the mother plants there do not seem to exist any constant positions from which the connecting bundles are to start. The bundles leaving the vascular rings of the mother plant for an adventitious bud are always inversely oriented, and, in most cases, become concentric as they grow near the bud. When the bud is provided with adventitious roots, the concentric bundles from the mother plant together with the bundles from the adventitious roots form, sometimes, two vascular rings at the basal region of the bud.

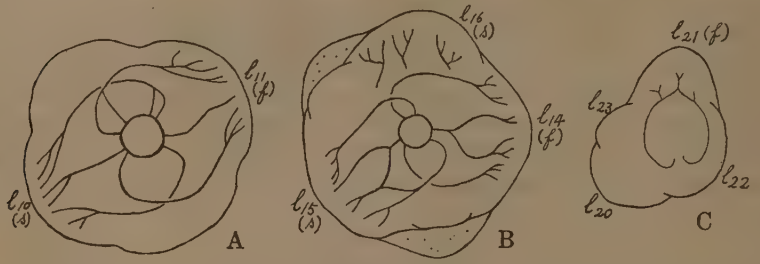
Leaf traces

As is already known, the leaf traces of *C. revoluta* assume a tangential and circular course through the cortex, and they are called girdle traces. METTENIUS (1861) observed that each leaf had two girdle traces, which joined together into a single bundle just before entering the central cylinder of the stem. On the other hand WORSDELL (1898), in his work on the anatomy of the seedlings of this plant, says that the cotyledons as well as the scale leaves have three traces each, and that their course is perfectly straight and radial in direction. I tried to reexamine the number and the course of the leaf traces, good and sufficient material being placed at my disposal.

1st cotyledon	3
2nd cotyledon	3
1st leaf ⋮ 13th leaf — all scale leaves	3
14th leaf — foliage leaf	3
15th leaf — scale leaf	3
16th leaf — „	3
17th leaf — „	2
18th leaf — „	2
19th leaf — „	2
20th leaf — foliage leaf	2
21st leaf — „	2
22nd leaf — scale leaf	2

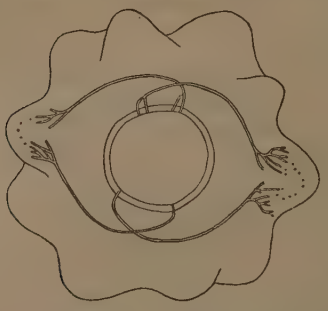
The traces of the cotyledons and those of the succeeding leaves were followed with a pincette and dissecting needle in several seedlings. The number of the traces was always three in the cotyledons as well as in the scale leaves. The data for seedling no. 2 (text-fig. 1, B) are shown in the table. In this seedling, the leaves are discernible up to the 31st leaf, but younger leaves than the 22nd leaf are not yet suitable for following their traces. Both cotyledons and the early formed leaves, whether they be scale leaf or foliage leaf, are supplied with three leaf traces; but the change of the number of traces begins at the 17th leaf, and younger leaves than this are all provided with two leaf traces. The

leaves with two leaf traces are lacking a trace corresponding to the middle trace of the early formed leaves, and show only the girdle traces (text-fig. 13, C). In the leaves with three leaf traces the middle trace sometimes divides into two bundles soon after it has left the central cylinder (text-fig. 13, B). This is also the case in the cotylendons (text-



Text-fig. 13. Leaf traces in seedling no. 2. $l_{10}(s)$ designates the 10th leaf which is scaly, $l_{11}(f)$ the 11th leaf which is foliar. Explanation in the text. ×1.

fig. 4, A). The traces of the cotyledons and of a few succeeding leaves are not conspicuous in girdling (text-fig. 4), but these girdle traces nevertheless start from the central cylinder on the opposite side of the leaf which they enter, and pass through the cortex over one third of the circumference of the stem before entering the leaf (text-fig. 13). Besides, in text-fig. 13, A, there are found two short radial bundles which run between the central cylinder and the girdle traces. These bundles, like the leaf traces, are of primary origin and already known by the name of radial connections (Radialverbindungen, Verbindungszweige). Though radial connections occur only rarely in the seedling, several connections adding to a single trace are often found in adult plants.

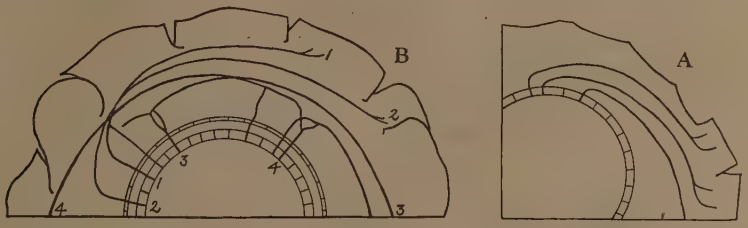


Text-fig. 14. Girdle traces and radial connections in the smaller, asexually produced plant. ×1/2.

In the large plants above-mentioned the discs 1 cm. or more thick were cut from different heights of the stem, and the course of the leaf traces was followed in the discs. The traces are all girdle traces and the number of the traces belonging to a leaf is two, no matter whether the leaf is a foliage leaf, a scale leaf, or a sporophyll (text-fig. 14). Generally the

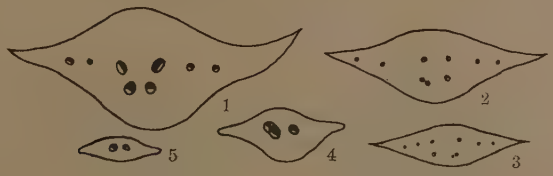
girdles, after leaving the central cylinder, ascend slightly in a radial direction, but they soon run nearly horizontally.

Text-fig. 15, A, shows three girdle traces found near the top of a large stem. The angle between both ends of a girdle is variable even in the girdles of neighbouring leaves. Text-fig. 15, B shows girdle traces found in a stem disc cut from the larger, asexually produced plant



Text-fig. 15. Girdle traces. A, near the top of a large stem; B, at the lower part of the stem of the larger, asexually produced plant. ×1/2.

above-mentioned. The angle of a girdle is about 90° in girdle 1, about 135° in girdle 2, about 150° in girdle 3, and about 140° in girdle 4. It is noticed that girdle 2, though it starts from the central cylinder on the left hand of girdle 1, enters a leaf situated on the right hand of the leaf which girdle 1 goes into. Such an irregularity renders it difficult to find out the rule which may exist concerning the arrangement of the girdle traces.

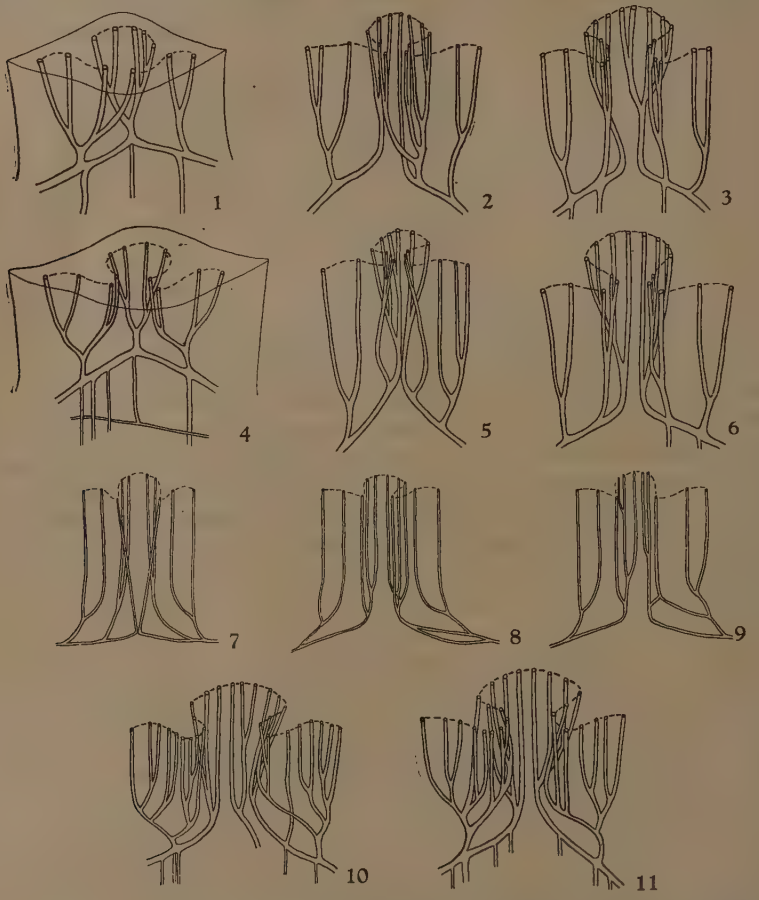


Text-fig. 16. Cross sections at the bases of the leaves. 1, foliage leaf; 2-3, scale leaves; 4, sporophyll with seeds; 5, sporophyll with abortive seeds. ×2.

The course and the number of the leaf traces were also observed in the larger of the two buds produced on the plant shown in text-fig. 9. The condition of two girdle traces begins from the first leaf and that of three traces as found in seedlings is quite lacking in this bud.

As mentioned above, all kinds of leaves are provided with two girdle traces in adult plants. However at the entrance into the leaf the girdles behave differently in different kinds of leaves or even in the same kind

of leaf. In the sporophyll, generally, either of the two girdles divides to form a branch, the two branches thus formed join together to form the median bundle of the sporophyll shaft, and there are found three bundles at a short distance above the base of the sporophyll shaft. Scale



Text-fig. 17. Girdle traces at the basal regions of the leaves. 1–6, foliage leaves from a large plant; 7–9, scale leaves from the same plant; 10–11, foliage leaves from the larger, asexually produced plant. $\times 1^1/_2$.

leaves present two kinds of shapes, one is thick and short and the other thin and long. The girdles of the thin scales behave as those of the sporophylls at their entrance into the scales. The foliage leaves and the thick scale leaves resemble each other closely in the manner in which

their girdles branch, but differ remarkably in the thickness of their girdles. Text-fig. 16 shows transverse sections through the bases of leaves of different kinds.

Though the girdle-branching at the leaf base is the same in principle in the case of foliage leaves as well as of scale leaves, different kinds of modifications often occur. Text-fig. 17 shows some instances in the branching of the girdles; 1–6 are the branching found in the foliage leaves of a 90 cm. long female plant, 7–9 in the scale leaves of the same plant, and 10–11 in the foliage leaves appearing at the top of the larger, asexually produced plant above-mentioned. In most cases the girdles of a leaf do not join together, and one of the girdles gives off two branches at the base of the leaf (text-fig. 17, 6). But other cases occur, in which both girdles join together into a bundle, or in which only one branch arises from the girdle (text-fig. 17, 1 and 4). Sometimes one of the two girdles of a leaf sends out only one branch, while the other gives off two branches (tex-fig. 17, 2). Intermediate cases are often found, too. It is interesting to find a bundle between the two girdles in text-fig. 17, 10, but it is not certain whether this bundle is quite independent from the two girdle traces, while further observations are needed to determine whether or not the condition of three leaf traces can really occur in adult plants.

Cortical concentric bundles

In *Cycas revoluta*, one can find an abundance of cortical concentric bundles of secondary origin, which run through the cortex in a longitudinal direction, forming, together with the girdle traces, a lattice work. Upper ends of these are connected at the bases of the leaves with the girdles or with the branches of the latter. This fact was made clear by METTENIUS (1860), and even in the seedling WORSDELL (1898) affirmed the presence of such bundles, but there nevertheless remain some points in need of further investigation.

These concentric bundles enclose a small amount of parenchyma in their centers; and in sections they are easily distinguishable from the leaf traces and radial connections because of their concentric constitution in contrast to the collateral structure of the latter. Besides, a fundamental difference exists between the cortical bundle and the leaf trace or the radial connection, because the former is differentiated secondarily, while the latter are of primary origin.

The course of the concentric bundles was observed at the bases of the foliage leaves, which were growing around the female cone of a large plant. Some of the leaves are shown in text-fig. 17, 1–6. The

number of the concentric bundles belonging to a leaf is most frequently 0–4. Older leaves lying some 10 cm. below the growing point are also provided with 0–4 concentric bundles. As regards the scale leaves of the same plant it is only rarely possible to find one or two concentric bundles joined to the girdles of a leaf. Some of the scale leaves within the female cone are also provided, though rarely, with concentric bundles of slight thickness. It seems that the cortical concentric bundles are formed comparatively early in the process of development of the leaf.

The concentric bundles starting from the girdles of a leaf run down the cortex of the stem and their lower ends terminate also on girdle traces; often it is found that they join together by twos or threes into thicker concentric bundles soon after they have left the girdles. Among the concentric bundles starting from the bases of different leaves, also, joining and branching very frequently occur. Besides, within the lattice work formed by the cooperation of the girdles and the cortical concentric bundles there are often found very thin concentric or semiconcentric bundles, which connect neighbouring girdles.

If one observes the crossing points in the lattice work, it will be noticed that the concentric bundles are often pierced through by the girdles, and that in such cases the concentric bundles usually divide just below the crossing points into two thinner concentric bundles. The cortical bundles are generally concentric, but they often give off very thin branches which are of collateral constitution. As regards the inner structure of the cortical concentric bundles, bundles or parts of bundles of small diameter are quite lacking the medullary parenchyma, while thick bundles enclose some amount of parenchyma within them.

The general features of the cortical concentric bundles have been mentioned above, but sometimes some modifications are found. The lower ends of the cortical bundles generally end on girdles at different levels of the stem; but, when I examined a stem segment, 24 cm. long and 18 cm. thick, taken from a large, male plant, it was noticed that all the cortical bundles passing down from the leaves produced within a certain length of the segment ended nearly at the same level, and this set of leaves was thought to have grown in the same growing season. The entire length of this segment consisted of a few sets of leaves of this nature, the length of a set being about 5 cm. Then, the system of the cortical concentric bundles in this segment consisted of a few sets of bundles. It may be added that in this stem segment the concentric bundles arising from the base of a leaf were counted as 3–7.

It can be shown that the cortical concentric bundles are quite independent from the development of the successive rings of wood and bast, and that their lower ends are connected with girdle traces. However, in the upper part of a thick stem (pl. III, fig. 4) some of the concentric

bundles were found open near their lower ends and were found entering the first secondary ring so as to form some segments of the ring, while others were found divided at their lower ends into thinner bundles which adhered also to the first secondary ring, while the rest of the concentric bundles terminated on girdle trace in the usual manner. In text-fig. 18, the course of the concentric bundles as just mentioned is shown semi-diagrammatically: one bundle joins the first secondary ring (*2*) and the other ends on a girdle trace (*t*). Besides, as regards the concentric bundles going down from the bases of two neighbouring leaves it was often found that the bundles from one leaf joined to the first secondary ring, while those from the other ended on girdle traces; and even as regards the concentric bundles from one and the same leaf it seemed that some bundles entered the first secondary ring, while the rest terminated on girdle traces.

Text-fig. 18. Unusual course of the cortical concentric bundles found near the top of a thick stem shown in pl. III, fig. 4. *1*, normal vascular ring; *2*, first secondary vascular ring, upper end of which is shown by *e*; *t*, leaf trace; *c*, cortical concentric bundle. Lower end of one cortical concentric bundle joins on to the first secondary ring, while that of the other bundle connects with a girdle trace. ×1.

The cone dome

It is already known that cone domes appear in the stem in certain genera of Cycadaceae; and as regards the genus *Cycas*, CHAMBERLAIN (1935) says that there are no cone domes in the female plant, but that cone domes should appear in the male plant. In accordance with what he says, the present species shows cone domes in the stem of the male plant, which is sympodial, but the female plant is quite lacking in cone domes, its stem being quite monopodial. In the upper part of a male plant shown in pl. III, fig. 4 three successive cone domes may be seen.

Summary

1) In the seedling of *Cycas revoluta* the first secondary ring of wood and bast is continuous through the stem and the tap root, and not interrupted in the transition region. A further anomalous structure exists; namely a vascular ring of normal orientation occurs in the transition region between the normal and the first secondary ring. This ring, higher up, divides into a few segments, which join on to the lower side of the cotyledonary plate or to the central cylinder of the stem. In the opposite direction some of the segments of the ring fuse with the central

cylinder, while the rest turn into concentric bundles, which pass down the root until they grow smaller and at last quite disappear. It is thought, for a certain reason, that this anomalous structure is formed as early as the central cylinder.

2) In the plant raised from an adventitious bud, the central cylinder of the stem is divided at the stem base into several parts. Each part enters one of the adventitious roots and forms there one or two vascular rings besides the central cylinder. This is why far more vascular rings are formed in the basal part of the stem than in any other part of it. The successive vascular rings, in the true sense, become differentiated outside the vascular system just mentioned.

3) An adventitious bud and its mother plant are connected in most cases by a few bundles, which become concentric as they come near the base of the bud.

4) In the seedling the cotyledons and the following leaves have three leaf traces, while the leaves formed later are provided with two leaf traces.

5) Generally the lower ends of the cortical concentric bundles terminate on leaf traces, however in one instance it was found that their lower joined on to the first secondary ring.

Botanical Institute, Faculty of Science,
Tokyo Imperial University.

References

Chamberlain, C. J. 1919. The living cycads. Chicago.

——— 1935. Gymnosperms. Structure and evolution. Chicago.

Coulter, J. M. and Chamberlain, C. J. 1925. Morphology of Gymnosperms. Chicago.

Dorety, Helen A. 1909. The extrafascicular cambium of *Ceratozamia*. Bot. Gaz. **47**, 150.

Gregg, W. H. 1887. Anomalous thickenings in roots of *Cycas Seemanni* Al. Br. Ann. of Bot. **1**, 63.

Hill, T. G. and de Fraine, E. 1909. On the seedling structure of Gymnosperms. III. Ann. of Bot. **23**, 433.

Mettenius, G. 1860. Beiträge zur Anatomie der Cycadeen. Abh. K. Sächs. Wiss. **7**, 565.

Miller, W. L. 1919. Polyxylic stem of *Cycas media*. Bot. Gaz. **68**, 208.

Smith, F. G. 1907. Morphology of the trunk and development of the microsporangium of cycads. Bot. Gaz. **43**, 187.

Solms-Laubach, H. 1890. Die Sprossfolge der *Stangeria* und der übrigen Cycadeen. Bot. Zeitg. **48**, 177.

South, F. W. and Compton, R. H. 1908. On the anatomy of *Dioon edule*, Lindl. New Phytol. **7**, 222.

Thomas, E. N. 1907. A theory of the double leaf-trace found on seedling structure. New Phytol. **6**, 77.

Worsdell, W. C. 1896. The anatomy of the stem of *Macrozamia* compared with that of other genera of Cycadeae. Ann. Bot. **10**, 601.

——— 1898. The comparative anatomy of certain genera of the Cycadaceae. Journ. Linn. Soc., Bot. **33**, 437.

——— 1900. The comparative anatomy of certain species of *Encephalartos*, Lehm. Trans. Linn. Soc. London, ser. 2, **5**, 445.

Explanation of plate III

Figs. 1–2. Three-year-old seedlings. (Cf. text-fig. 1). × 1/2.

Fig. 3. A plant raised from an adventitious bud. (Cf. text-fig. 9). × 1/3.

Fig. 4. Median longitudinal sections through the upper part of a thick stem. *1*, normal vascular ring; *2*, first secondary vascular ring; *c*, cortical concentric bundles; *d*, cone dome. (Cf. text-fig. 18). × 1/4.


1
2
3
c
1
2
d
d
d
4

Studies on the polyploidy in *Nicotiana* induced by the treatment with colchicine

I. General observations on the autotetraploid of *Nicotiana rustica* and *N. Tabacum*

By Yakiti NOGUTI, Kikuo OKUMA and Hideto OKA
(Central Research Institute, Japanese Government Monopoly Bureau)

With 4 text-figures

(Received September 9, 1939)

Polyploidy has recently become one of the most attractive subjects of genetists because of the development of methods by which it can be artificially induced. Since JØRGENSEN (10) obtained the polyploid tomato by the so-called decapitation-callus method, a few workers have succeeded in causing the chromosome doubling of plant by the treatment with extreme temperature (6, 24, 27). Such physical methods, however, are not always promising as those of general applicability, though the decapitation-callus method has been proved to be pretty effective for promoting the formation of abundant calli and polyploid shoots, owing to the heteroauxin treatment of the cut surface (8, 9). As well known, BLAKESLEE and AVERY (1, 2, 3) have however discovered a more powerful method of increasing the number of chromosome sets by the use of drug colchicine, and since then its great efficacy in this respect has been proved by many investigators (5, 11, 13, 15, 17, 18, 19, 20, 21, 25, 28, 30, 31, 32, 33). The observations reported here refer to the autotetraploids of *Nicotiana* induced by colchicine.

Materials and methods

Three varieties of *Nicotiana, N. rustica* var. *brasilia, N. Tabacum* var. *Kokubu* and *N. Tabacum* var. *Yellow Orinoco,* were selected as materials in this experiment. To induce the polyploidy, the immersion of seeds in different concentrations from 0.05 to 1.6 per cent aqueous solution of colchicine for six days was done. For each treatment about

one hundred seeds were used. The immediate effect of the treatment was a general retardation of development, as seen in the slow germination and growth of seedlings, even when the doses were so weak as to bring about no visible changes in the adult plants. The swollen stem and poor rooting indicated externally spontaneous chromosome increase in the early cotyledonary stage. Such malformations were observed abundantly after the treatment with concentrated solutions and often led to the death of seedlings. The external judgement of tetraploid plants founded on their special roughened and dark green leaves, which are apt to be associated with spontaneous tetraploid, could succeed to some extent in the six- or eight-leaved stage, though of course the fact must finally be confirmed by the examination of pollen grain and the chromosome counts. The treatment with colchicine within a wide range of concentration is effective in inducing plants with double chromosome number, as given in Tab. 1.

TABLE 1. Effects of colchicine upon the seeds of *Nicotiana* immersed in its solution.

Varities	*N. rustica* var. *brasilia*			*N. Tabacum* var. *Kokubu*			*N. Tubacum* var. *Yellow Orinoco*		
Percentage of colchicine	Number of seeds	Percentage of germination	4n-plants	Number of seeds	Percentage of germination	4n-plants	Number of seeds	Percentage of germination	4n-plants
1.6	93	26	—	100	63	3	100	65	4
0.8	90	24	—	100	81	13	100	67	7
0.4	80	18	—	100	87	6	100	58	4
0.2	102	28	1	100	64	6	100	72	3
0.1	75	39	7	100	71	4	100	81	5
0.05	67	48	3	100	95	1	100	65	—
Cont.	—	—	—	100	93	—	60	70	—

In *N. rustica* the six days treatments with the solution above 0.4 per cent gave no evidence of its efficacy, while slightly weak solution induced often the tetraploidy. In the case of *N. Tabacum*, on the contrary, a number of tetraploid plants appeared in all the solutions of colchicine used, though especially the result of treatment with 0.8 per cent was most distinct.

Experimental results

(1) MORPHOLOGICAL CHANGE. The induced tetraploids which were extremely rubst were provided with thick rigid stems and much thickened

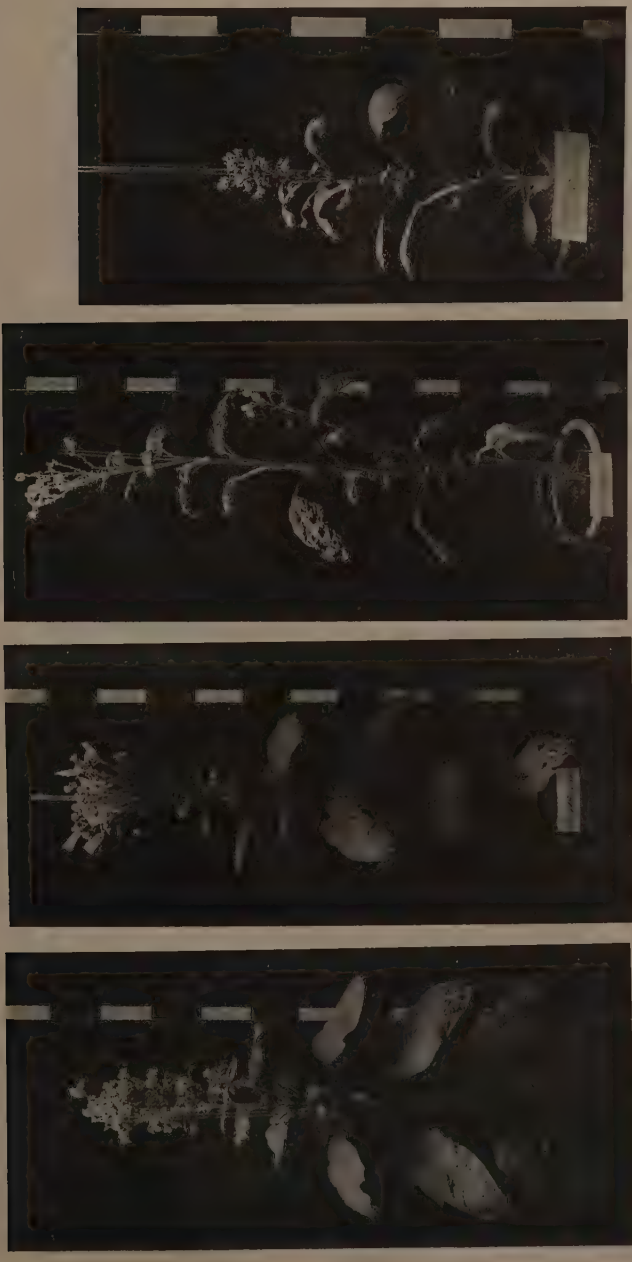


Figure 1. *Nicotiana rustica*. (a) A diploid plant; (b-d) three tetraploid plants produced by the application of colchicine, one of which (d) was completely sterile.

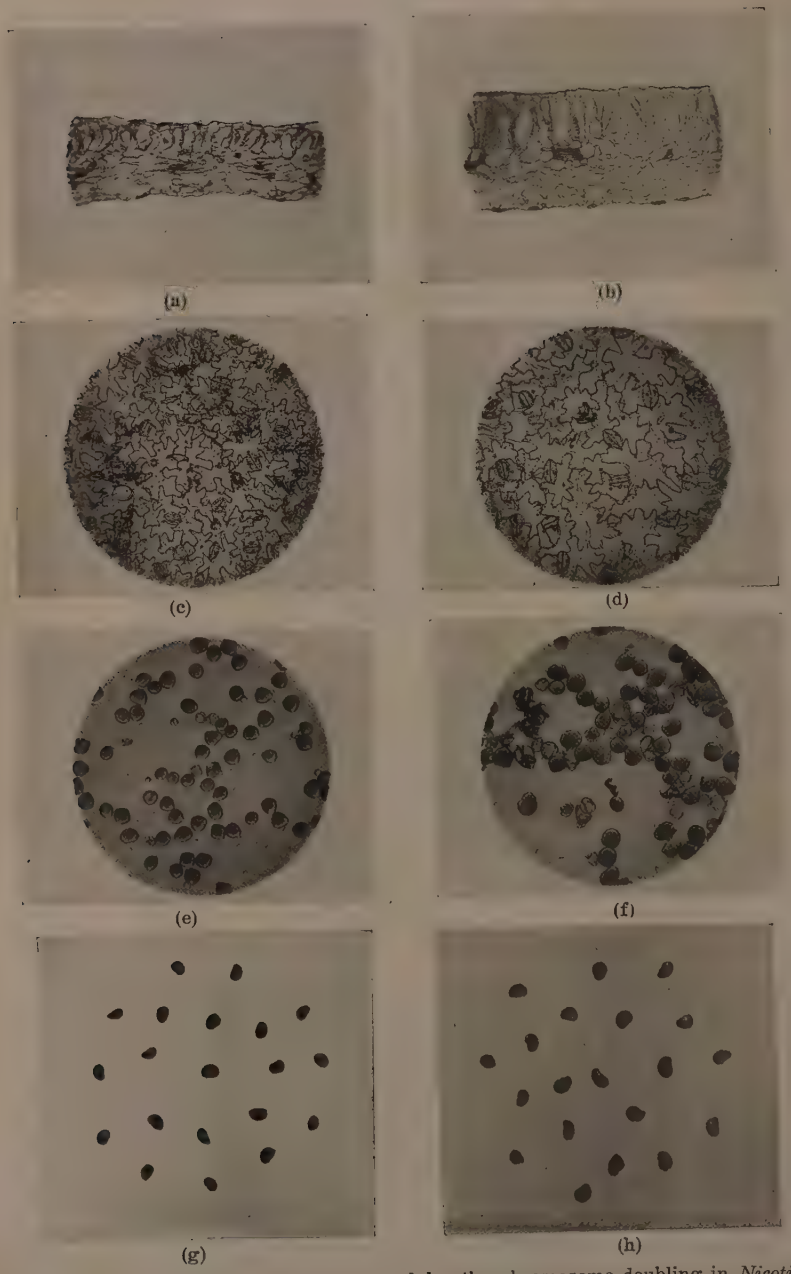


Figure 2. Morphlogical changes caused by the chromosome doubling in *Nicotiana rustica*: diploid on left and tetraploid on right. (a–b) section of leaves, (c–d) stomata and epidermal cells; (e–f) pollen grains and (g–h) seeds.

dark green leaves, which sometimes are fasciated and often had irregularly chewed-appearing surface (Fig. 1). As leaves developed, they indicated somewhat round shape and were not large as those of diploids. The extraordinary increase of leaves in thickness is shown in Tab. 2 (Fig. 2 a, b).

TABLE 2. Thickness of leaves.

Varieties	*N. rustica* var. *brasilia*		*N. Tabacum* var. *Kokubu*		*N. Tabacum* var. *Yellow Orinoco*	
	Thickness (mm)	Ratio	Thickness (mm)	Ratio	Thickness (mm)	Ratio
Diploid	0.350	100	0.244	100	0.320	100
Tetraploid	0.590	168	0.376	154	0.369	115

The enlargement of stomata and epidermal cells and the decrease of stomata number were also the characteristics of the tetraploids under discussion (Tab. 3) (Fig. 2 c, d).

TABLE 3. Number and size of stomata (*N. rustica*).

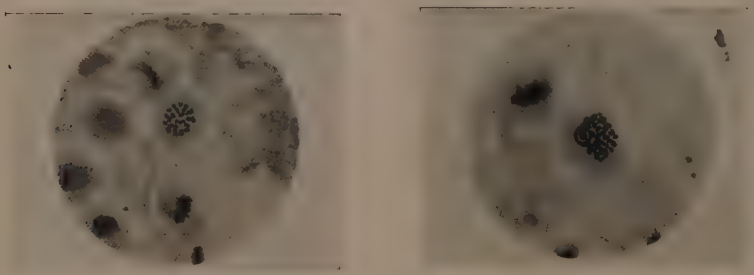
	Number of stomata*	Size of stomata	
		Length (mm)	Width (mm)
Diploid	28.4	0.38	0.31
Tetraploid	13.0	0.59	0.41

* The average of ten microscopic fields.

Even when the plants were completely developed, the tetraploids proved to be not so large as the diploids. They exhibited the delay in time of flowering, about two weeks in *N. rustica* and two months in *N. Tabacum*. The overall dimensions of their flowers were greater, but the capsule size was much reduced, owing to a small number of seeds contained therein. The pollen and seeds from the tetraploids were also larger than in diploids (Fig. 2 e-h). Some plants, which indicated afterwards an extreme dwarfness with wrinkled leaves were completely sterile (Fig. 1 d).

(2) CYTOLOGICAL BEHAVIOUR. Diploid plants of both *N. rustica* and *N. Tabacum* have 48 (2n) chromosomes and the reduction division of pollen mother cells goes on quite normally, producing good pollen. An examination of 1010 pollen grains proved that 802 grains or 79.4 per cent are normal. In sharp contrast to the behaviour of nuclear division

in diploids stands that in tetraploid, in which tetravalent, trivalent and univalent chromosomes appeared very often in the first metaphase stage (Fig. 3). Tetravalent chromosomes, at most 10–14, were connected longitudinally or formed the ring . There was no 4n pollen mother cell containing only bivalent chromosomes. On anaphase the chromosomes failed to be distributed normally, owing to the appearance of lagging chromosomes, and 50, 49, 47 or 46 chromosomes travelled to each pole. The pollen was found to be only about 39.6 per cent good, i.e. 397 in 1003 grains. The fertility of tetraploid is reduced extraordinarily; for instance, only 10–15 capsules containing 10–15 seeds or none at all were got usually on one tetraploid *rustica* stock, in contrast to the diploid one producing 60–80 capsules, each containing 500–600 seeds.



(a) (b)

Figure 3. Polar view of the first meiotic metaphase of *Nicotiana rustica*; (a) diploid and (b) tetraploid.

(3) NICOTINE CONTENT. Besides the great morphological and cytological changes, the doubled chromosome number brings about often certain biochemical ones in the plant organism. An increase from 51 to 138 per cent of nicotine content was found in the tetraploid *Nicotiana* plants as compared to diploids, as shown in Tab. 4.

TABLE 4. Nicotine content.

Varieties	*N. rustica* var. *brasilia*		*N. Tabacum* var. *Kokubu*		*N. Tabacum* var. *Yellow Orinoco*	
	Nicotine content (%)**	Ratio	Nicotine content (%)	Ratio	Nicotine content (%)	Ratio
Diploid	1.240	100	2.133	100	2.530	100
Tetraploid	2.980	238	3.456	162	3.840	151

* The percentage of dry matter.

** The average for three leaves directly above ground (*N. rustica*) and in all leaves (*N. Tabacum*).

It may safely be said that the tetraploidy causes the increase of nicotine content in N. rustica and N. Tabacum.

Discussion

There are a number of methods that are known to affect the mechanism of cell division and produce polyploid plants artificially. Of these methods, until recently the treatments by extreme temperature were considered to be most promising, though the percentage of success was not very high in spite of difficult techniques (24, 27). On the other hand, the decapitation-callus method performed by JØRGENSEN (10) was recognized to be an important one, because the simple cutting operation induced the production of 10–20 per cent tetraploid plants in tomato (10, 14, 22). That method has been thereafter applied to several kinds of plant, but the percentage of tetraploid produced thereby was very low, only 1–2 per cent, even in the case of success. Quite recently GREENLEAF (8, 9) has obtained 10–20 per cent tetraploids in *Nicotiana* by putting heteroauxin on the surface of cuttings, so such decapitation-callus method was considered as a useful method of inducing polyploids. By the discovery of the use of colchicine, however, a new method has been started quite lately that promises to be of wide use in the production of polyploid plants in large percentage than in any other method. This was indicated at first by the work of BLAKESLEE and AVERY (1, 2, 3), and many investigators have proved its efficacy. In our experiments the use of colchicine has shown also to be highly efficacious in inducing an abundance of tetraploids in *Nicotiana*. Six days treatment of seeds with colchicine within a fairly range of concentration was effective, of which the most efficacious was 0.1 per cent and 0.8 per cent towards *N. rustica* and *N. Tabacum* respectively. WARMKE and BLAKESLEE (33) reported already that they obtained 51 per cent polyploid plants by treating seeds of *Nicotiana Sanderae* with 0.2–0.8 per cent colchicine solution and 13 per cent polyploids of F_1 (*N. Tabacum* × *N. glutinosa*) by spraying from an atomizer 1 per cent colchicine solution mixed with lanolin on the growing point. SMITH (30) has also induced the production of many polyploids by the treatment of seed with 0.2–0.8 per cent colchicine solution and by the application of 0.4 per cent colchicine in water solution of lanolin paste in *Nicotiana* species and species hybrids. As far as known till now, the soaking method of seeds is most effective in *Nicotiana* for inducing polyploidy with colchicine, and it may, moreover, be improved by a suitable combination of proper concentration and treatment. On the other hand, the temperature was recognized to act an important rôle upon the treatment of seeds in the authors experiment. The hypocotyl

elongated abnormally and died away soon after the germination, when the temperature is too high. So it is necessary to keep the seed under such a moderate temperature as scarcely to allow the development of the radicle.

It is generally said that the polyploid plants are very often more robust, with thicker stem and larger leaves, flowers and seeds than in diploids (16). The autotetraploids raised in our experiment were however seen to grow slowly, had smaller and thicker leaves, and were, except a few, just as large as the undoubled forms, when the plants are completely developed. They had also undergone such morphological change as the swelling of hypocotyls, the enlargement of pollen and stomata, which were noticed by BLAKESLEE and AVERY (2) as the characteristics of polyploid. SMITH (30) recently reported in his observation of autotetraploids in *Nicotiana Tabacum* and *N. rustica* that they were smaller with smaller and thicker leaves than the diploids. A similar type of growth was also found by the authors in the culture of tetraploid *rustica* under the glass house condition in winter (Fig. 4). So that the quantity of solar radiation may be a powerful factor for the growth of tetraploids.

Figure 4. A tetraploid of *Nicotiana rustica*, which much decreased in size under the glass house condition in winter.

The autotetraploids in *Nicotiana* induced by colchicine were commonly reported to be almost sterile (30, 33), and the appearance of tetravalent chromosomes in the first metaphase of pollen mother cells is regarded as its main cause, but this is quite questionable because many natural polyploids as well as the tetraploid cabbage made by SHCHAVINSKAYA (29) experimentally show normal nuclear division of pollen mother cells and yet set many seeds, even with the appearance of tetravalent chromosomes. MÜNTZING (16) stated that the sterility of polyploids induced artificially is due to their physiological conditions, which, however, he did not make clear. The sterility of polyploid is an important question for the practical breeding, so it ought to be studied more extensively in future.

Not only the morphological but also the change of another category takes place after the chromosome doubling, thus for instance the reduction of cell-sap concentration reported by WETTTSTEIN (34) in the moss

and seen by SCHLÖSSER (27) in the tomato. The increase of chemical substance content in the cell was also seen in several cases; thus OKA (23) observed extraordinary increase of sugar, organic acid and vitamin C[1] in the tetraploid tomato. Quite recently, KOSTOFF (12) has measured alkaloid and citric acid in the leaf of *Nicotiana glauca*, *N. Langsdorffii* and their amphidiploid hybrids and has found that their content in the hybrids varied very much and was often more than in the parental forms. In the tetraploids *Nicotiana rustica* and *N. Tabacum* a great increase of nicotine was seen by the authors in our experiment as above noticed, and this is perhaps one new instance of the increase in chemical substance content due to the chromosome doubling. In all, the precise study of the physiological characters in polyploids should be an important item in future.

Summary

(1) In order to induce polyploidy, the seeds of *Nicotiana rustica* var. *brasilia*, *N. Tabacum* var. *Kokubu* and *N. Tabacum* var. *Yellow Orinoco* were treated with different concentration of colchicine (0.05 to 1.6 per cent)for six days.

(2) In *N. rustica* 0.1 per cent solution was most effective and in *N. Tabacum* the result of treatment was most distinctly seen at 0.8 per cent. Sixty-seven tetraploids were raised in total.

(3) The tetraploid plants, the judgment of which by their external appearance could be successfully done in the six or eight leaves stage, were in general extremely robust with much thickened dark green leaves. The enlargement of stomata and epidermal cells and the decrease of stomata number were also their characteristics.

(4) The retardation of growth in tetraploids is associated with the delay of their flowering time. The overall dimensions of their flowers and seeds were, however, greater than those of diploids.

(5) The reduction division of pollen mother cells goes on quite normally in the undoubled form, consequently good pollen is produced. The behaviour of nuclear division in the tetraploid is, however, disturbed by the appearance of tetravalent, trivalent and univalent chromosomes in the first metaphase. Their pollen was found to be only 39.6 per cent functional. The fertility of tetraploid plants is also reduced extraordinarily.

(6) A considerable increase of nicotine content is observed in the tetraploids as compared to that of diploids. Especially, in *N. rustica* the rate of increase was about 138 per cent.

(1) About vitamin C CRANE and ZILVA (4), SANSOME and ZILVA (26) and GÖTHLIN (7) have found already the same tendency in the apple.

Literature cited

(1) BLAKESLEE, A.F. and AVERY, A.G. 1937. Methods of inducing chromosome doubling in plant by treatment with colchicne. Science **86**: 408.

(2) ——— 1937. Methods of inducing doubling chromosomes in plants. Journ. Hered. **28**: 395–411.

(3) BLAKESLEE, A.F., AVERY, A.G. and CARTLEDGE, J.L. 1938. Induction of polyploid in *Datura* and other plants by treatment with colchicine (Abst. 1937 Meet. Gen. Soc. Amer.). Genetics **23**: 140–141.

(4) GRANE, M.B. and ZILVA, S.S. 1931. The antiscorbutic vitamin of apples. Journ. Pomol. and Hort. Science. **9**: 228–231.

(5) DERMEN, H. 1938. A cytological analysis of polyploidy induced by colchicine and by extremes of temperature. Journ. Hered. **29**: 211–229.

(6) DORSEY, E. 1936. Induced polyploidy in wheat and rye. Journ. Hered. **27**: 155–160.

(7) GÖTHLIN, G.F. 1935. Biologiska bestämningar av halten av antiskörbjuggsvitamin (vitamin C, askorbinsyra) hos 12 av de viktigaste i Sverige odlade äpplesorterna (with a summary in English). Kungl. landtbruks-Akademiens Handlingar och Tidskrift. **7**: 884–944.

(8) GREENLEAF, W.H. 1937. Induction of polyploidy in *Nicotiana*. Science **86**: 565–566.

(9) ——— 1938. Induction of polyploidy in *Nicotiana*. Journ. Hered. **29**: 451–464.

(10) JØRGENSEN, C.A. 1928. The experimental formation of heteroploid plants in the genus *Solanum*. Journ. Genet. **19**: 133–211.

(11) KIHARA, H. and KISHIMOTO, E. 1938. Erzeugung polyploider Individuen durch Colchicin bei *Celosia cristata* (in Japanese with a summary in German). Agric. and Hort. **12**: 2623–2628.

(12) KOSTOFF, D. 1938. Studies on polyploid plants. XXI Cytogenetic behaviour of the allopolyploid hybrids *N. glauca* GRAH. × *N. Langsdorffii* WEINM. and their evolutionary significance. Journ. Genet. **37**: 129–209.

(13) LEVAN, A. 1938. The effect of colchicine on root mitosis in *Allium*. Hereditas **24**: 471–486.

(14) LINDSTROM, E.W. and KOOS, K. 1931. Cytogenetic investigations of a haploid tomato and its diploid and tetraploid progeny. Amer. Journ Bot. **18**: 398–410.

(15) MORRISON, G. 1938. A tetraploid *Zinnia*. Journ. Hered. **29**: 187–188.

(16) MÜNTZING, A. 1936. The evolutionary significance of autopolyploidy. Hereditas **21**: 263–378.

(17) NEBEL, B.R. 1937. Cytological observations on colchicine. Biol. Bull. **73**: 351–352

(18) ——— 1937. Mechanism of polyploidy through colchicine. Nature **140**: 1206–1208.

(19) ——— 1938. Colchicine and acenaphthene as polyploidizing agents. Nature **142**: 257.

(20) NEBEL, B.R. and RUTTLE, M. L. 1938. The cytological and genetical significance of colchicine. Journ. Hered. **29**: 3–9.

(21) NISHIYAMA, I. 1938. Polyploid plants induced by the colchicine method (in Japanese with an English résumé). Bot. and Zool. **6**: 1404–140.

(22) OKA, H. 1938. Studies on the polyploidy in tomato. I. The fertility of tetraploid plants (in Japanese). Journ. Hort. Ass. Japan **9**: 231–254.

(23) ——— 1939. On the physiological characters of autotetraploid in tomato (in Japanese). Bot. and Zool. **7**: 461–464.

(24) RANDOLPH, L.F. 1932. Some effects of high temperature on polyploidy and other variation in Maize. Nat. Acad. Sci. Proc. **18**: 222–229.

(25) SANDO, W.J. 1939. A colchicine induced tetraploid in buckwheat. Journ. Hered. **3**: 271–272.

(26) SANSOME, F.W. and ZILVA S.S. 1933. Polyploidy and vitamin C. Biochem. Journ. **27**: 1935–1941.

(27) SCHLÖSSER, L.A. 1936 Frosthärte und Polyploidie. Züchter **8**: 75–80.

(28) SCHWANITZ, F. 1938. Die Herstellung polyploider Rassen bei *Beta* Rüben und Gemüsearten durch Behandlung mit Colchicin. Züchter **10**: 278–279.

(29) SHCHAVINSKAYA, S.A. 1937. Tetraploid cabbage obtained by means of regeneration. Bull. Appl. Bot. Gen. and Pl.-br. **11** (7): 32.

(30) SMITH, H.H 1939. Induction of polyploidy in *Nicotiana* species and species hybrids by treatment with colchicine (Abst. 1938 Meet. Gen. Soc. Amer.). Genetics **24**: 85–86.

(31) WALKER, R.T. 1938. The effect of colchicine on microspore mother cells and microspores of *Tradescantiai paludosa*. Amer. Journ. Bot. **25**: 280–285.

(32) WARAMKE, H.E. and BLAKESLEE, A.F. 1939. Effect of polyploidy upon the sex mechanism in dioecious plants (Abst. 1938 Meet. Gen. Soc. Amer.). Genetics **24**: 88–89.

(33) ——— 1939. Induction of tetraploidy in *Nicotiana Sanderae* and in the sterile hybrid *N. tabacum* × *N. glutinosa* by colchicine treatment (Abst. 1938 Meet. Gen. Soc. Amer.). Genetics **24**: 109–110.

(34) WETTSTEIN, F. v. 1924. Morphologie und Physiologie des Formwechsels der Moose auf genetischer Grundlage. I. Zeitschr. f. ind. Abst-. und Vererb. -Lehre. **33**: 1–236.

Influence of physical and chemical factors upon the formation of appressoria in the conidia of *Piricularia Oryzae*

I. Influence of oxygen[1]

By Hashio SUZUKI

With plate IV

(Received September 14, 1939)

It has been confirmed by a number of investigators (1, 2, 3, 4, 6, 7) that the germ tube of the conidium of *Piricularia Oryzae* BR. et CAV., the rice blast fungus, can not directly penetrate host plant, and that its ability to do so is restricted to the penetrating hypha that develops from the appressorium which is formed on the end of the germ tube (Plate IV, Fig. 1). It may, therefore, be said that appressorium formation is closely related to entrance of the fungus into the host plant through its conidia.

SUETA (4) first reported that the germ tubes of the fungus conidia can form appressoria only through contact stimulus. Recently, ITO and SHIMADA (2), who support SUETA's conclusion, also pointed out that the germ tubes of the fungus conidia are able to form appressoria by contact with a glass surface.

The writer, from experiments made on the factors influencing the formation of appressoria in the conidia of *P. Oryzae*, found that, in addition to contact stimulus, physical and chemical factors also play an important part in appressorium formation. This paper gives the results of the experiments in so far as they relate to the part played by oxygen in the formation of appressoria, as reported at the annual meeting of the Phytopathological Society of Japan, April 1939 (5).

Methods and results:—It was found from a number of experiments that the fungus conidia are able to form appressoria on the surface of

(1) This work was supported in part by a grant from the Imperial Academy, to which the writer here records his grateful thanks. The fungus used in this work was isolated from the interior of the rice seed.

the cellophane, that is conveniently used for the microscopical examinations by the writer, as well as on that of glass after germination in distilled water or in one per cent solution of glucose, and that the last-named solution is more suited for germination and appressorium formation than distilled water. For these reasons, cellophane and one per cent solution of glucose were used throughout all the experiments.

Two small pieces of cellophane, about two square cm, were placed in each of the Petri dishes and then sterilized. A few drops of the glucose solution, in which the fungus conidia were suspended, were placed in the center of each piece of cellophane. About five c.c. of the same solution were then gradually poured into the dishes in such a manner that the pieces of cellophane with a few drops of spore suspension floated on the surface of the solution. The oxygen concentration was adjusted by reducing the content of oxygen in the air, using definite quantities of potassium hydroxyde (10%) and pyrogallic acid (1%), assuming that the concentration of oxygen in the air is 20 per cent. The oxygen concentrations, that were, consequently, approximate, were 0, 5, 10, 15, 17, 18, 19, and 20 per cent. Accordingly, the reduction of pressure which occurred in the concentration of 0 to 19 per cent was out of consideration in the writer's experiments.

As soon as the definite quantities of potassium hydroxyde and pyrogallic acid have been mixed in the bottom of the desiccator (content 1,700 c.c.), the Petri dishes, containing the small pieces of cellophane with a few drops of spore suspension floating on the surface of the glucose solution, were placed in the upper part of the desiccator, and covered to make it air-tight. The desiccator was then placed in a thermostat, the temperature of which was maintained at 25°C. After incubation for 48 hours, the germination and formation of appressoria were microscopically examined.

The fungus used in the experiments, which was isolated from the interior of rice seed (Omati) in May 1934, was cultured on potato agar to which was added one per cent of sucrose at 25°C for from two to three weeks. The experiments were made during the period from October 1938 to May 1939.

The results are shown in Table 1. The figures in the table showing the concentration of oxygen are not accurate, they being more or less variable.

It will be seen from Table 1 that when the oxygen was completely removed from the air, the conidia of *P. Oryzae* were not able to germinate and, consequently, could not form any appressoria (Plate IV, Fig. 2). Germination of the conidia was observed in oxygen concentrations of 5, 10, 15, 17, 18, 19, and 20 per cent (Plate IV, Figs. 3–8). The percentage of germination was very much lower in concentration

TABLE 1. Influence of oxygen on the germination and formation of appressorium in the conidia of *Piricularia Oryzae*.

Experiment	Concentration of oxygen (%)	Number of conidia tested (A)	Number of condia germinated		Number of appressoria formed	
			Number of germinated conidia counted (B)	Percentage (B/A)	Number of appressoria counted (C)	Percentage (C/A)
I	0	450	0	0	0	0
	10	450	440	97.78	0	0
	15	500	492	98.40	0	0
	17	450	445	98.89	0	0
	18	400	394	98.50	378	94.50
	19	400	396	99.00	402	100.50
	20	450	448	99.56	475	105.56
II	0	380	0	0	0	0
	5	300	96	32.00	0	0
	10	300	294	98.00	0	0
	15	350	348	99.43	35	10
	17	300	296	98.67	154	51.33
	18	350	346	98.86	255	72.86
	20	300	298	99.33	314	104.67
III	0	530	0	0	0	0
	5	450	125	27.78	0	0
	10	480	473	98.54	0	0
	15	445	441	99.10	0	0
	18	550	545	99.09	405	73.64
	20	500	498	99.60	552	110.40

of 5 percent than in 10, 15, 17, 18, 19, and 20 per cent; it was almost the same in 10, 15, 17, 18, and 19 per cent as in 20 per cent. The length of the germ tubes in 5 and 10 per cent concentrations is very much shorter than that in the other concentrations, showing a severe inhibition of the growth of germ tubes owing to the insufficient supply of oxygen (Plate IV, Figs. 3–8). Appressorium was formed without exception throughout all the experiments in 18, 19, and 20 per cent concentrations, but not in those of 5 and 10 per cent (Plate I, Figs. 3–8). The percentage of apressoria formed in oxygen concentrations of from 18 to 19 per cent is almost the same as that in 20 per cent, the former being somewhat smaller than the latter. Appressorium formation was not positively determined in concentrations of 15 and 17 per cent, the reasons being that in the former concentration a small number of appressorium developed only in Expt. II, while in the latter, it developed abundantly in Expt. II, but none in Expt. I.

Judging from the results of the experiments above described, a severe inhibition of germination of the fungus conidia seems to appear in oxygen concentration of 5 per cent, whereas the same of appressorium formation appears in that of 15 or 17 per cent. The minimum concentration of oxygen for appressorium formation seems to lie between 10 and 15 per cent and that for germination between 0 and 5 per cent.

As just mentioned, oxygen seems to be one of the most important factors in germination, especially, in the formation of appressoria in the conidia of *P. Oryzae*, and the amount of oxygen necessary for the formation of appressoria seems to be much greater than that necessary for germination.

Literature cited

1. IKATA, S., MATUURA, G., and TAGUTI, S.: Bureau of Agr., Dept. Agr., Nozikairyosiryo, No. **20**, pp. 1–140, 1931.
2. ITO, S., and SHIMADA, S.: Ibid, No. **120**, pp. 1–109, 1937.
3. MATUURA, G.: Journ. Plant Protect., **15**, pp. 571–574, 1928.
4. SUETA, H.: Dept. Agr., Central Res. Inst., Govern. Formosa, Bull. No. **36**, pp. 1–130, 1928.
5. SUZUKI, H.: Ann. Phytopath. Soc. Japan, **9**, p. 119, 1939 (Abstract).
6. YOSHII, H.: Journ. Plant Protect., **20**, pp. 841–844, 1933.
7. YOSHII, H.: Ann. Phytopath. Soc. Japan, **6**, pp. 205–218, 1936.

Explanation of plate IV

Fig. 1. Appressoria formed on the ends of germ tubes of the conidia of *Piricularia Oryzae*, × ca. 850.

Figs. 2–8. Appressorium formation in different concentrations of oxygen, × ca. 485.

Fig. 2. Oxygen completely removed; Fig. 3, 5 per cent; Fig. 4, 10 per cent; Fig. 5, 15 per cent (Expt. I); Fig. 6, 17 per cent; Fig. 7, 19 per cent; Fig. 8, 20 per cent.

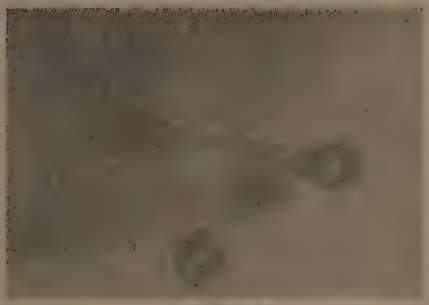


Fig. 1

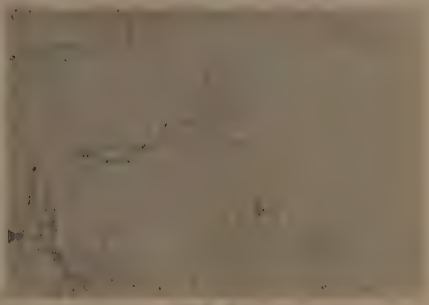


Fig. 2

Fig. 3

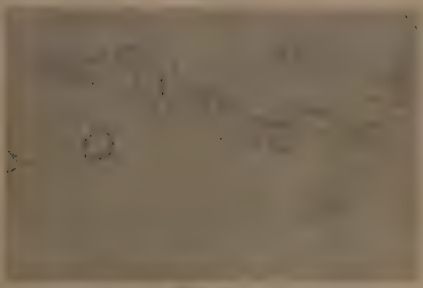


Fig. 4

Fig. 5

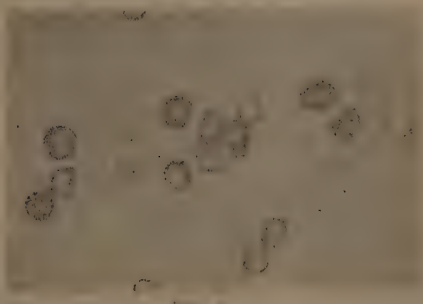


Fig. 6

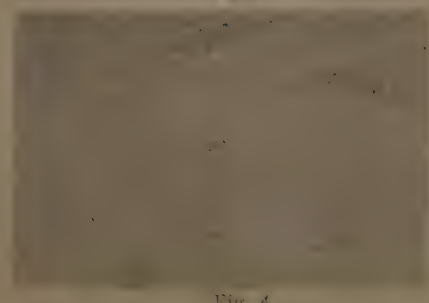


Fig. 7

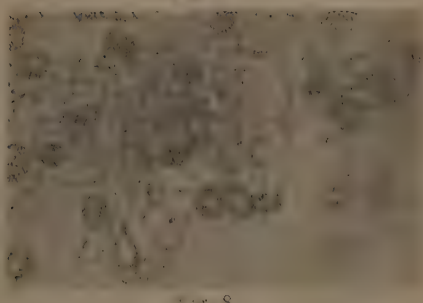


Fig. 8

Judging from the results of the experiments above described, a severe inhibition of germination of the fungus conidia seems to appear in oxygen concentration of 5 ... cent, whereas the same of appressorium formation appears in that of ... 17 per cent. The minimum concentration of oxygen for appressorium formation seems to lie between 10 and 15 per cent and that for germination between 0 and 5 per cent.

As just mentioned, oxygen seems to be one of the most important factors in germination, especially in the formation of appressoria in conidia of *P. Oryzae*, ... amount of oxygen necessary for the formation of appressoria ... to be much greater than that necessary ... germination.

Literature cited

1. KATAYAMA, G., and ... Bureau of Agr., ... Agr., Nozikairyoziryo, No. **20**, pp. ... 130, 1931.
2. ITO, S., and SHIMADA, S.: Ibid., No. **120**, pp. 1–109, 1937.
3. ... Journ. Plant ... 15, pp. 571–574, 1928.
4. ... : ... Inst., Govern. Formosa, Bull. No. **36**, pp. 1–130, 1928.
5. SUZUKI, H.: Ann. Phytopath. Soc. Japan, **9**, p. 119, 1939 (Abstract).
6. YOSHII, H.: Journ. Plant Protect., **20**, pp. 841–844, 1933.
7. YOSHII, H.: Ann. Phytopath. Soc. Japan, **6**, pp. 205–218, 1936.

Explanation of plate IV

Fig. 1. Appressorium formed on ... ends of germ tubes of ... conidia of *Pyricularia Oryzae*. × ca. 850.

Figs. 2–8. Appressorium formation in different concentrations of oxygen, × ca. 850.

... removed. ... 3, 5 per cent; ... per cent; Fig. 5, 15 per cent (Expt. I); Fig. 6, 17 per cent; Fig. 7, 19 per cent; Fig. 8, 20 per ...

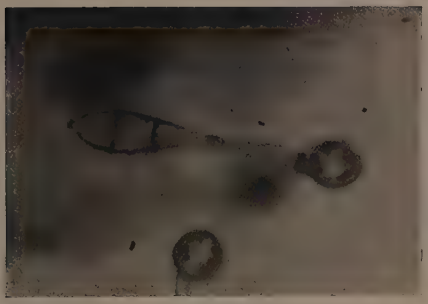


Fig. 1

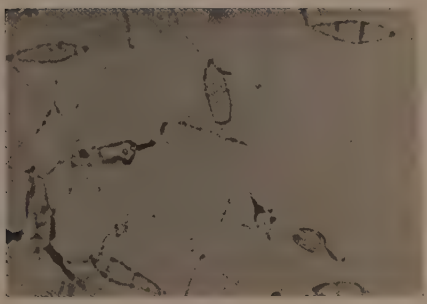


Fig. 5

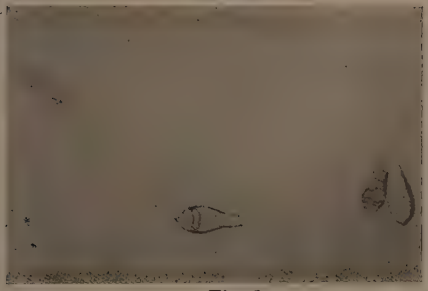


Fig. 2

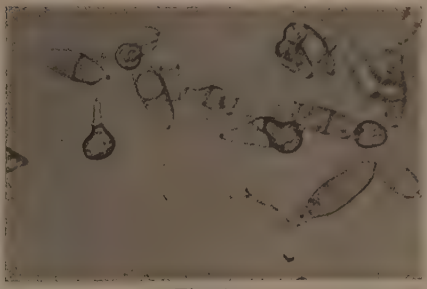


Fig. 6

Fig. 3

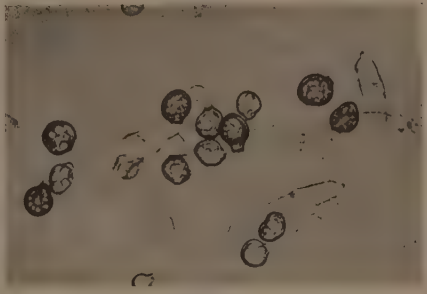


Fig. 7

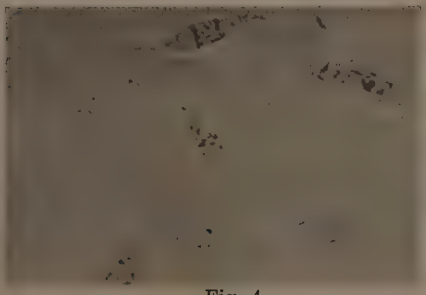


Fig. 4

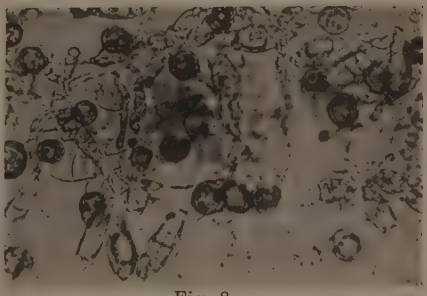


Fig. 8

Studies on the formation of ascorbic acid (vitamin C) in plants

2. The influence of radiation of different wave-lengths on the ascorbic acid contents in etiolated seedlings

By Tomota SUGAWARA

With 4 text-figures and 1 table

(Received September 20, 1939)

Introduction

In the previous experiment (17) the author has announced the fact that the etiolated seedlings exposed to low light intensity contained much less ascorbic acids than those exposed to high light intensity, and that the differences in the ascorbic acid content of seedling under the two light conditions were attributable to the difference of light intensities. Further, it was stated that when seedlings containing chlorophyll were exposed to light, ascorbic acid is formed in their leaves, so that a direct relationship between the photosynthesis and the quantity of ascorbic acid may be quite probable.

The effect of light of different wave-lengths upon the carbon dioxide assimilation has been the subject of investigation by a considerable number of plant physiologists during the last 150 years. According to their results, the efficiency of the photosynthetic mechanism in green plants decreases, in most cases, with the decreasing wave-lengths. Recently, DASTER and MEHATA (5) studied the photosynthetic activity under red, blue-violet, and day light of equal intensities, and have found that it was highest in white light, lower in red, and still lower in blue-violet. It was further proven that both the red and the blue-violet regions of the spectrum are necessary for the normal photosynthetic activity, and even under white light the assimilation depends on the combination of radiation of different wave-lengths.

Since, according to the author's opinion, the direct relationship between the formation of ascorbic acid and photosynthesis should exist, he thinks that it will be very interesting to ascertain whether light of

different wave-lengths will play by itself an active part on the formation of ascorbic acid or not. The present investigation was undertaken by the author for casting a certain light on the solution of the question.

Experimental procedure

The plants used in these experiments were maize, pea, soybean, Chinese cabbage (*Brassica pekinensis* RUPR), *Phaseolus vulgaris* L., radish, barley, and oat. The procedure employed to get etiolated seedlings has been outlined in the previous paper (17). Seeds were germinated on moist quartz-sand or filter-paper, and after germination the seedlings were kept in the dark room at about 20°C for one week before exposure to light of different wave-lengths. Vigorous etiolated seedlings were divided into six groups, and these plants were exposed to visible spectrum

Fig. 1. General arrangement of the apparatus.

of different regions for 24 or 48 hours respectively, and then the seedlings, whose root was removed away, were analyzed for total ascorbic acid (reduced form plus oxidized form of ascorbic acid). The quantity of the ascorbic acid was determined by the titration method with 2,6-dichlorphenolindophenol solutions described by EMMERIE and FUJITA et al. (8, 9).

The source of light used was the gas-filled lamps (1000, 500, and 200 watt) of ordinary type. Six plots of plants to be experimented upon, including the control, were radiated by red (6700– — Å), orange-red (6450– — Å), green (5600–4550Å), blue (5050–3800Å), and white light. A series of filters was made by covering the glass plate by dyed gelatin films, and it was mounted on a filter cell cooled by means of tap-water or cold air current. Fig 1 shows the arrangement of water-cooled cell

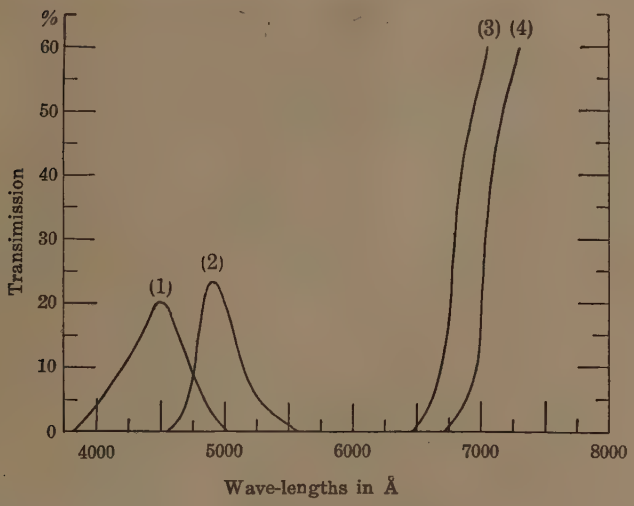


Fig. 2. Transmission of filters used in wave-lengths series; (1) blue, (2) green, (3) orange-red, (4) red.

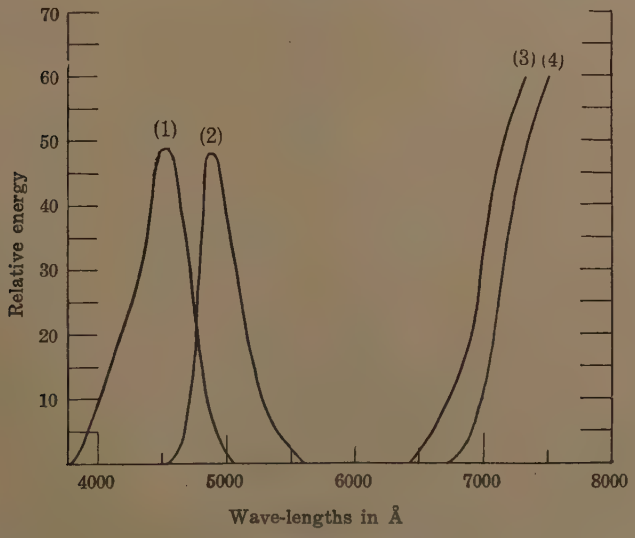


Fig. 3. Energy distribution curves of filter combinations; (1) blue, (2) green, (3) orange-red, (4) red.

for filtering the radiation of electric lamps. The transmission spectra of the water cells and the color filters were measured with a BAUSCH and LOMB spectrophotometer and a thermopile, and the energy distribution was calculated from HOLLANDY, BARNES and FORSYTHE tables (1, 10), by assuming average color temperatures of 2980°K for 1000 watt MAZDA lamps, 2920°K for 500 watt lamps, and 2810°K for 200 watt lamps. The energies in the various bands were sufficiently balanced so that the effects secured could hardly be attributable to difference of intensity. The relative energy distribution of the radiation emitted by the filter system for the lamps employed is plotted in fig. 3., which was obtained by multiplying the transmittancy given in fig. 2. The transmission bands of the filters were not of the same width, so it was obviously impossible to keep the total energy and the peak values equal for them. Then it was considered more significant to maintain the peak values of the same magnitude rather than the total energy. Since organic dyes are relatively unstable to light, the filters were checked for any change in transmission in each experiment and replaced with new ones, when needed.

Experimental results

The data obtained by the determination of the ascorbic acid content in various seedlings under the different regions of visible spectrum are given in table 1. The results of the analysis have shown that the red and orange-red portions had the greatest influence on the ascorbic acid contents of the seedlings than the blue and green. The fact was most distinctly seen in Chinese cabbage, soybean, and radish, while in maize and pea it was much less conspicuous. Considerable variation was found in the different species as regards the amount of ascorbic acid. In most species the ascorbic acid content of seedlings was particularly high in the red portion, which in peas, barleys, etc. it was slightly lower under the red than under the orange-red (fig. 4). Although there is an indication of slightly greater formation of ascorbic acid in the orange-red portions than in the red, the differences between the two are not statistically significant. When the etiolated seedlings of maize and peas are exposed to different regions of visible spectrum, the amount of ascorbic acid increased much less than in any other species. In radish, the results did not coincide with the general observations on the ascorbic acid content, and the percentage of the acids was relatively high in the green and blue portions of spectrum . The reason of such a variation may be found in the fact that the seedlings of radish under green and blue light were growing vigorously at the time of analysis. In most

TABLE 1. Effect of radiation of different wave-lengths on ascorbic acid contents in seedlings.

Plants	Predominating color and wave-lengths (Å)	24 hours		48 hours	
		Ascorbic acids contents mg./g.	Increase by radiation %	Ascorbic acids contents mg./g.	Increase by radiation %
Zea Mays L. (Long Fellow)	Red (6700– —)	0.543	10.5	0.540	11.1
	Orange-red (6450– —)	0.548	11.6	0.540	11.1
	Green (5600–4550)	0.530	7.9	0.536	10.2
	Blue (5050–3800)	0.512	4.2	0.515	5.9
	White ———	0.555	13.0	0.565	16.2
	Dark (control)	0.491	—	0.486	—
Pisum sativum L. (Alaska)	Red (6700– —)	0.730	14.0	0.740	13.4
	Orange-red (6450– —)	0.728	13.7	0.744	14.1
	Green (5600–4550)	0.685	7.0	0.697	6.9
	Blue (5050–3800)	0.664	3.7	0.690	5.8
	White ———	0.743	16.0	0.746	14.4
	Dark (control)	0.640	—	0.652	—
Glycine Max. MERE. (Turunoko)	Red (6700– —)	0.451	59.3	0.456	63.4
	Orange-red (6450– —)	0.460	62.5	0.460	64.8
	Green (5600–4550)	0.372	31.4	0.380	36.2
	Blue (5050–3800)	0.361	27.5	0.374	34.0
	White ———	0.455	60.7	0.463	65.9
	Dark (control)	0.283	—	0.279	—
Brassica pekinensis RUPR. (Chihli Pe-tsai)	Red (6700– —)	0.765	61.3	0.765	64.5
	Orange-red (6450– —)	0.656	38.3	0.701	50.7
	Green (5600–4550)	0.613	29.3	0.610	31.1
	Blue (5050–3800)	0.590	24.4	0.616	32.4
	White ———	0.930	96.2	0.958	106.0
	Dark (control)	0.474	—	0.465	—
Phaseolus vulgaris L. (Satisfaction)	Red (6700– —)	0.568	26.5	0.573	26.2
	Orange-red (6450– —)	0.550	22.4	0.566	24.6
	Green (5600–4550)	0.484	7.7	0.507	11.6
	Blue (5050–3800)	0.490	9.1	0.509	12.1
	White ———	0.622	38.5	0.640	40.9
	Dark (control)	0.449	—	0.454	—
Raphanus sativus L. var. *macropoda* (Minowase)	Red (6700– —)	0.826	35.1	0.850	41.1
	Orange-red (6450– —)	0.798	30.6	0.855	42.0
	Green (5600–4550)	0 815	33.3	0.829	37.7
	Blue (5050–3800)	0.810	32.5	0.820	36.2
	White ———	1.063	73.6	1.045	73.5
	Dark (control)	0.611	—	0.602	—
Hordeum vulgare L. (Sekitori)	Red (6700– —)	0.305	23.9	0.313	25.2
	Orange-red (6450– —)	0.307	24.7	0.324	29.6
	Green (5600–4550)	0.301	22.4	0.300	20.0
	Blue (5050–3800)	0.290	17.8	0.295	18.0
	White ———	0.353	43.4	0.362	44.8
	Dark (control)	0.246	—	0.250	—
Avena sativa L. (Race horse)	Red (6700– —)	0 681	36.7	0.683	40.8
	Orange-red (6450– —)	0.670	34.5	0.648	33.6
	Green (5600–4550)	0.646	29.7	0.647	33.4
	Blue (5050–3800)	0.600	20.4	0.602	24.1
	White ———	0.745	49.5	0.760	56.7
	Dark (control)	0.498	—	0.485	—

cases, the highest quantity of ascorbic acids corresponding to each wave-length was found after 48 hours radiations.

It was evident from the data above mentioned that the quantity of ascorbic acid present in the seedlings under the different regions of visible spectrum varies in the following order: white light > red portion ≧ orange-red portion > green portion > blue portion.

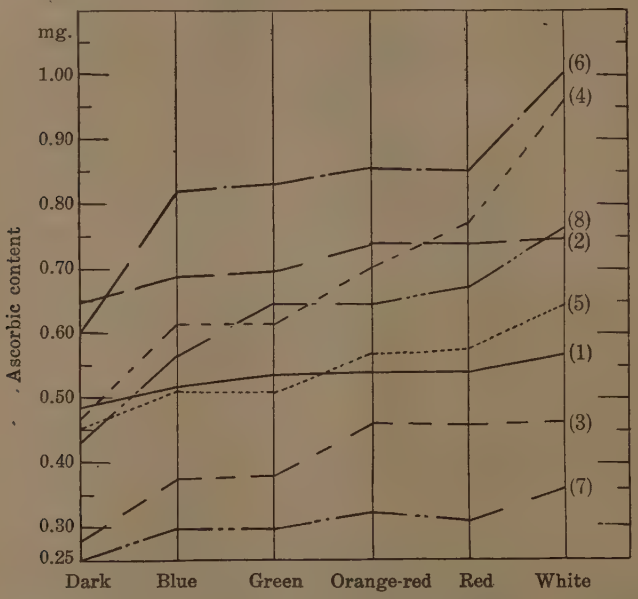


Fig. 4. Relation between the wave-lengths and ascorbic acid content in seedlings; (1) maize, (2) pea, (3) soybean, (4) Chinese cabbage, (5) Chaseolus, (6) radish, (7) barley, and (8) oat.

Discussion

In the experiment just described on seedlings exposed to different regions of visible spectrum an increased formation of ascorbic acid was found, as compared with those placed in dark during the same duration of time, all other external conditions being kept as uniform as possible, so that it may be safely concluded that the radiation of spectrum plays an important part in the production of ascorbic acids. Regarding the formation of ascorbic acids the white light was most effective and in this respect follow the red, the orange-red, the green, and the blue light in descending order. Even under the red light, however, the production of ascorbic acid was less than under white light, and it may be also clear

that both the red and the blue portions take their respective part in the formation of ascorbic acid.

It was shown rather conclusively that the photosynthesis proceeds more rapidly at the red than at the blue-violet end (LUBIMENKO and others), in fact, the careful work of URSPRUNG (18) has shown it to be true even when both regions were kept under equal energy value. URSPRUNG and NEGELEIN (19, 20) have found, that the efficiency of photosynthetic system decreases with the decreasing wave-lengths, and BRIGGS (2) came also to the same conclusion. BURNS (3) has studied the quantum yields in monochromatic as well as white light of equal intensity, and confirmed the results obtained by BRIGGS. On the other hand, WURMSER (21, 22) concluded that green light is utilized in photosynthetic process about four times as the red light. The experiment of POPP (12) proved, however, that the blue-violet region is essential for the formation of carbohydrates in leaves. DASTER and MEHATA (5) have recently worked with the red, blue-violet, and white light of equal intensities and found that a larger quantity of carbohydrate was formed in the leaves in the red light than in blue-violet, and the highest amount was formed in the white light. So generally speaking the efficiency of the photosynthesis in green plants decreases with the decreasing wave-lengths. DASTUR and others (6) have found moreover, that the whole of visible spectrum is photosyntheticaly effective, and the photosynthetic activity was highest in white light, lower in red light, and still lower in blue-violet light, as above noticed.

The light intensities and qualities, the amount of chlorophyll, and the concentrations of carbon dioxides are usually considered to be important factors concerning the photosynthetic activities. In the previous experiment, the authors (17) found that the amount of ascorbic acid in the seedlings is directly proportional to the increase of light intensity, at least within the limits of his experiments. It was found also that when seedlings containing chlorophyll are exposed to light ascorbic acid increases in their leaves, while the albino seedlings under illumination indicates no increase of ascorbic acid just as in the seedlings kept dark during the same time duration. The present investigation proves, moreover, that a considerable amount of ascorbic acid was formed in seedlings even under red light, while the greatest amount of these substances was produced under white light.

In all, it may be concluded that the formation of ascorbic acid is closely connected with the photosynthetic system. Similar results were got by RANDOIN et al. (13, 14) who found the fact that the ascorbic acid content of leaves increases when irradiated by neon-light and that etiolated plants contain no vitamin C. On the other hand, CLARKE (4) has reported that the distribution of ascorbic acid in coleoptile corresponds

to that of chlorophyll. From the results of these experiments, it may certainly be stated that the formation of ascorbic acid and the photosynthetic mechanism stand in close physiological interrelationship.

Summary

1. The effect of artificial radiation by light of different wave-lengths on the ascorbic acid contents in etiolated seedlings was studied. The plants used were maize, pea, soybean, radish, etc., and these plants were divided into six plots, including the control, and radiated with red (6700– — Å), orange-red (6450– — Å), green (5600– 4550 Å), blue (5050–3800 Å), and white light.

2. The formation of ascorbic acid in the seedlings is affected by the action of radiation of different wave-lengths. A considerable amount of ascorbic acid is produced, even in the blue and green portions, but its production is more considerable under red and white light. The quantity of ascorbic acid present in the seedlings under different regions of visible spectrum were in the following order: white light > red portion ≧ orange-red portion > green portion > blue portion.

Acknowledgement

I wish to acknowledge my deep indebtedness to Prof. Y. NOGUTI, and express my cordial thanks for the keen interest always taken by him as well as his valuable criticism throughout the progress of this investigation and the preparation of this paper.

LABORATORY OF AGRONOMY, FACULTY OF AGRICULTURE,
TOKYO IMPERIAL UNIVERSITY

Literature cited

(1) BARNES, B.T. and FORSYTH, W.E.: Spectral radiat intensities of some tungsten filament incandescent lamps. Jour. Opt. Soc. Amer. **26**: 313, 1936.

(2) BRIGGS, G.E.: Experimental researches in vegetable assimilation and respiration. XX. The energetic efficiency of photosynthesis in green plants; some new data and discussion of the problem. Proc. Roy. Soc., B **105**, B 734, 1, 1929.

(3) BURNS, G.R.: Photosynthesis in various portions of the spectrum. Plant Physiol. **8**: 247, 1933.

(4) CLARK, W.G.: Ascorbic acid in the *Avena* coleoptile. Bot. Gaz. **99**: 116, 1937.

(5) DASTUR, R.H. and MEHTA, R.J.: The study of the effect of blue-violet rays on photosynthesis. Ann. Bot. **49**: 809, 1935.

(6) ———, and SOLOMAN, S.: A study of the effect of blue-violet rays on the formation of carbohydrates in leaves. Ann. Bot. N. S. **1**: 147, 1937.

(7) DUGGAR, B.M.: Biological effects of radiation. Vol. **I, II**, 1936.

(8) EMMERIE, A. and VON EEKELEN, M.: The chemical determination of vitamin C with removal of interfering reducing and coloured substances. Biochem. Jour. **28**: 1153, 1934.

(9) FUJITA, A. u. EBIHARA, T.: Kolorimetrische Bestimmung von Vitamin C mittels Phospho–18 Wolfram Säure I. Bestimmung von Gesamt- Vitamin C. Biochem. Z. **290**: 192, 1937.

(10) HOLLANDAY, L.L.: Proportion of energy radiated by incandescent solids in various spectral regions. Jour. Opt. Soc. Amer. **17**: 329, 1928.

(11) LUBIMENKO, V.: Action spécifique des rayon lumineux de diverses couleurs dans la photosynthèse. Compt. Rend. Acad. Sci. Paris, **177**: 606, 1923.

(12) POPP, H.W.: A physiological study of the effect of light of various ranges of wave-length on the growth of plants. Amer. Jour. Bot. **13**: 706, 1926.

(13) RANDOIN, L. GIROUD, A. et LEBLON, C.P.: Relation entre la teneur en vitamine C de divers tissus végétaux et la présence ou l'absence de chlorophylle. Compt. Rend. Soc. Biol. Paris, **120**: 297, 1935.

(14) ———, ———, et ———: Recherches biologiques et biochemiques sur la teneur en acide ascorbique des tissus chlorophylliens et achlorophylliens. Bull. Soc. Chem. Biol. Paris, **17**: 1649, 1935.

(15) SUGAWARA, T.: Influence of fertilizers on the ascorbic acid content of spinach. Jour. Soc. Soil and Manure, Japan, **12**: 567, 1938.

(16) ———,: Studies on the ascorbic acid content in the spinach. Jour. Hort. Asso. Japan, **10**: 189, 1939.

(17) ———,: Studies on the formation of ascorbic acids in plants 1. Influence of light on the ascorbic acid contents in various etiolated seedlings. Jap., Jour. Bot. **10**: 141, 1939.

(18) URSPRUNG, A: Über die Stärkebildung im Spectrum. Ber. Deutsch. Bot. Ges. **35**: 44, 1917.

(19) WARBURG, O. und NEGELEIN, E.: Über den Energieumsatz bei der Kohlensäureassimilation. Zeitschr. f. Physikal. Chem. **105**: 235, 1922.

(20) ———, und ———,: Über den Einfluss der Wellenlänge auf den Energieumsatz bei der Kohlensäureassimilation. Zeitschr. Physiol. Chem. **106**: 191, 1933.

(21) WURMSER, R.: L'action des radiations de différentes longueurs d'onde sur l'assimilation chlorophyllienne. Compt. Rends. Acad. Sci. Paris, **170**: 820, 1920.

(22) ———,: Sur l'activité des diverses radiations dans la photosynthèse. Compt Rend. Acad. Sci. Paris, **180**: 374, 1925.